Diatoms, dinoflagellates and their distinct effects on the structure and function of the bacterioplankton

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III. TC participated in the preparation and set-up of the experiment, daily sampling and sampling and measurements of the bacterioplankton variables, decision-making process and analysis and interpretation of the bacterial community composition results. TC contributed in writing of the manuscript in collaboration with KS.

LIST OF ABBREVIATIONS

ÅS	Åland Sea	DSi	Dissolved silica
ArS	Archipelago Sea	ENSO	El Niño Southern Oscillation
AT	*Achnanthes taeniata* treatment	HCS	The Humboldt Current System
ATP	Adenosine triphosphate	HMW	High molecular weight
BA	Bacterial abundance	HNF	Heterotrophic nanoflagellates
BB	*Biecheleria baltica* treatment	HTS	High-throughput sequencing techniques
Bp	Bases pare	GoF	Gulf of Finland
BCC	Bacterial community composition	Leu	Leucine
BCD	Bacterial carbon demand	LMW	Low molecular weight
BGE	Bacterial growth efficiency	NMDS	Non-metric multidimensional scaling
BoB	Bay of Bothnia	OMZ	Oxygen Minimum Zone
BP	Baltic Proper	OUT	Operational taxonomic unit
BPL	Bacterial production leucine	P	Phosphorous
BPT	Bacterial production thymidine	PCR	Polymerase chain reaction
BR	Bacterial respiration	PER	Percentage of extracellular release
BS	Bothnian Sea	PERMANOVA	Repeated measures permutational ANOVA
BSP	Bacterial secondary production	PP	Primary production
CO_2	Carbon dioxide	POC	Particulate organic carbon
Chl *a*	Chlorophyll *a*	POM	Particulate organic matter
CF	Conversion factors	N	Nitrogen
Dia : Dino index	Diatom/dinoflagellate index	NAG	N-acetylglucosamine
DINOF	Dinoflagellate treatment	rRNA	Ribosomal ribonucleic acid
DOC	Dissolved organic carbon	TB	*Thalassiosira baltica* treatment
DOM	Dissolved organic matter	TdR	Thymidine
		TEP	Transparent exopolymer particle

DIATOMS, DINOFLAGELLATES AND THEIR DISTINCT EFFECTS ON THE STRUCTURE AND FUNCTION OF THE BACTERIOPLANKTON

MARÍA TERESA CAMARENA GÓMEZ

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Global warming is one of the most alarming pressures affecting marine ecosystems worldwide. One of the indirect effects of the increasing surface-water temperature is the change in phytoplankton community composition, shifting in some ecosystems from diatom predominance towards the dinoflagellate predominance or co-occurrence with diatoms during blooms. These distinct phytoplankton groups vary in the quality and/or quantity of the dissolved organic matter (DOM) they release, shaping the bacterioplankton community composition and metabolism. The predominance of diatoms or dinoflagellates during productive events may have contrasting effects on the associated bacterioplankton communities, in terms of structure and function, and also on the carbon flux passing through the microbial loop, due to selective utilization of dissolved organic carbon (DOC) by different bacterial taxa.

The main objective of this thesis was to assess the effect of diatoms and dinoflagellates on shaping the bacterial community composition and dynamics in different ecosystems dominated by these distinct phytoplankton groups, such as the Baltic Sea and the Humboldt Current System (HCS) off Chile. This was achieved by conducting two experiments in the Gulf of Finland, four consecutive campaigns in the Baltic Sea during and after the spring bloom and one experiment in the central HCS off Chile. Further studies were conducted in the Baltic Sea to determine the bacterial productivity and abundance during different phases of the phytoplankton spring bloom.

Phytoplankton community composition and the stage of the bloom phase clearly affected to the bacterial community composition and dynamics in both ecosystems. Alphaproteobacteria, dominated by SAR11 and Rhodobacteraceae, was the most abundant bacterial class in all studies. The oligotrophic SAR11 dominated in pre-bloom conditions and was associated with dinoflagellates. In contrast, copiotrophic bacteria belonging to the classes Flavobacteriia, Gammaproteobacteria, Betaproteobacteria and the family Rhodobacteraceae (genera *Loktanella, Planktotalea, Planktomarina* and *Amylibacter*) were associated with diatom species such as *Achnanthes taeniata*, *Chaetoceros* spp., *Skeletonema marinoi* and *Thalassiosira levanderi* in the Baltic Sea and with *Thalassiosira* spp. in the HCS. In addition, in the Baltic Sea, bacterial communities dominated by copiotrophs had higher bacterial production rates than in SAR11 dominated bacterial communities. Hence, the diatom-released DOM boosted the development of more productive bacterial communities during phytoplankton blooms. Further differences in the bacterial community composition were driven by the different salinities in these two ecosystems; Betaproteobacteria, Planctomycetes and Actinobacteria were more abundant in the brackish Baltic Sea than in the HCS.

The results of this thesis highlight that the shift to the dinoflagellate dominance or co-occurrence with diatoms may affect to the bacterial community composition and activity during bloom events. Certain diatom species promotes the growth of copiotrophic bacteria, which contribute largely to high bacterial production rates and recycling of carbon. In contrast, the increase in dinoflagellate abundance associated with global warming may potentially change the pelagic remineralization of organic matter, which could reduce the carbon flux to higher trophic levels.

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CONTENTS

1 INTRODUCTION

1.1 Microbes in marine ecosystems

Marine ecosystems are governed by microbes, single-celled organisms, including autotrophic and heterotrophic prokaryotes, autotrophic and heterotrophic eukaryotes and viruses. In the case of prokaryotes, they are characterized by their small size (0.3-1.0 μm), large numbers (*i.e.* bacteria 10^6 cells per litre), fast growth and capability for occupying all types of environments (soil, fresh and salty waters), even under extreme conditions of pH, pressure or temperature (Karl 2007, Kirchman *et al.,* 2017). Moreover, these organisms play an important role in the biogeochemical cycles of nutrients, *e.g.* carbon, nitrogen (N), phosphorus (P) and sulphur, that affect the global climate (Falkowski *et al,.* 2008). Thus microbes, in general, and algae and bacteria in particular, are important components of the global ecosystem and key players in its functioning.

Microbes have a long evolutionary history, *i.e.* 3.700 million years ago (Nutman *et al.*, 2016), based on palaeobiological and molecular evidence. This antiquity has favoured the acquisition of traits, such as their ubiquity in changing ecosystems, along a geological scale. Thus, when Baas-Becking (1934) promulgated the famous statement "everything is everywhere, but the environment selects", he referred to the environment as the only important variable determining the abundance of a phylotype. This seems to match with the biogeographical distribution of small prokaryotes, but at the same time there are mismatches between the protistan communities identified by phylogenetic markers, such as ribosomal ribonucleic acid (rRNA) genes, and their geography (Kirchman 2012). This incongruence arise when looking at the scale of comparison, *e.g.* how the level of the phylotype identification using the 16S rRNA gene was defined since the identification can be done at 97%, 99%, or 100%. It is true that marine microbes, *i.e*. phytoplankton and bacterial communities, can be restricted by environmental disturbances, *e.g.* by annual (seasonal or spatial-temporal changes) and interannual, *i.e.* El Niño Southern Oscillation (ENSO) phenomena that can determine the patterns of diversity (Montecino *et al*., 2004). There are further abiotic factors affecting the bacterioplankton, such as temperature, salinity, oxygen level, pH, availability of inorganic nutrients and organic matter, but also biotic factors (grazing, viral attack). Those factors constitute bottom-up and top-down regulation of the structure and dynamics of the community (Fuhrman 1999, Joint *et al.* 2002, Vargas & González 2004, Legrand *et al.,* 2015, Bunse *et al.*, 2016, Bunse & Pinhassi 2017). These environmental disturbances can alter the microbial composition following three theoretical responses (*sensitivity, resistance*, and *resilience*), which are dependent on the intensity of the disturbance and on the capability for returning to initial conditions (Allison & Martiny 2008, Lindh and Pinhassi 2018). Thus, a community can be altered by an environmental disturbance, resulting in a community different from the original and being functionally dissimilar for some microbial taxa (*sensitivity*), one that may require a certain time (several years) until it returns to the pre-disturbance state (*resilience*) or one that is not affected by the environmental disturbance (*resistance*).

The challenge for scientists has always been the identification of microbes. Bacteria identification is especially challenging, due to their simple morphology and small size, whereas phytoplankton identification by microscopy is manageable. In addition, there were historical differences in the approach to addressing microbial ecology. As a first approach, 'the microbial autecology' focused on the isolation and identification of a single 'species', or bacterium, to understand its metabolic capabilities and relationship with the surrounding medium. However, problems emerged when the traditional technique, mainly cultivation of isolated cells on agar plates, was applied to the identification of marine bacteria, in contrast to the microscopy results. The plate count method underestimated the true bacterial numbers counted by epifluorescence microscopy, a discrepancy reported by Jannasch & Jones (1959). At the same time, heterotrophic bacteria were not considered to be part of the marine food webs, due to their role as simple decomposers of organic matter (Strickland 1965, Steele 1974). With the emergence of the microbial ecology approach in the 1960s, which combined the identification of single species with culture-independent methods and investigation of biogeochemical cycles, it became easier to identify the role of bacteria in marine food webs.

Microbial ecology has advanced rapidly in the phylogenetic identification field in recent decades, due to the development of molecular techniques (Pace 1997). The use of certain markers, such as the 16S rRNA gene, aided in the identification of natural microbial communities (Giovannoni *et al.,* 1990). The 16S rRNA is the most widespread marker used among bacteria for phylogenetic studies (Kirchman 2012). The success of this marker is due to the existence of the 16S rRNA genes in all bacteria and their highly conservative feature that allows the design of primers for targeting specific groups. Its length of 1,500 nucleotides is also appropriate for giving accurate taxonomic information, which was already observed in the 1980s in the analysis of mixed populations in natural samples (Pace *et al*., 1986). However, the 16S rRNA gene tool also has limitations, since the number of copies of this gene can vary among bacteria, and thus this technique is not quantitative. There may also be physiological and ecological/functional differences between two microbes with similar 16S rRNA genes or, in contrast, microbes closely related in terms of physiological or ecological responses can belong to different genera questioning the statement of "everything is everywhere" as explained above (Kirchman 2012).

Recently, sequencing techniques have improved rapidly from conventional first-generation techniques such as polymerase chain reaction (PCR), Sanger sequencing and fingerprinting (Fuhrman & Hagström 2008) to the development of high-throughput sequencing (HTS) techniques, such as 454-pyrosequencing, Illumina and Applied Biosystems SOLiD system platforms (Kircher & Kelso 2010, Metzker 2010, Liu *et al.*, 2012, Logares *et al.*, 2012). The success of the HTS for phylogenetic identification of microbial communities is the high throughput and continued decreasing cost. The HTS generates 10^6−10^9 sequences of 100−700 base pairs (bp) per run (Glenn 2011), which is achieved using massive parallel sequencing. In comparison, the Sanger technique may generate 10^2 sequences (Logares *et al.*, 2012). Thus, these platforms, and particularly Illumina, are frequently used in the identification of bacterial communities from different environments. However, the Sanger technique is still used for sequencing individual regions or single genomes due to the longer sequence length obtained (~ 900 bp).

1.2 The dissolved organic matter pool: the link between phytoplankton and bacterioplankton

The dissolved organic matter (DOM) pool in marine systems is the major organic carbon reservoir largely formed by non-living and refractory organic carbon (Falkowski & Raven 2007). This dissolved fraction can be collected by filtration, using 0.2 or 0.7 μm pore size filters. However, it is known that there are particles smaller than these sizes, *i.e.* viruses or other components (transparent gels or mucus) that pass through the filters and also form complex macromolecules within the dissolved fraction (Verdugo *et al.*, 2004). The DOM can be characterized by its molecular size or weight as low- (LMW) or high- (HMW) molecular weight (Amon & Benner 1996), its availability for degradation as labile, semi-labile or refractory (Keil & Kirchman 1999, Jiao *et al.,* 2010), its origin as autochthonous production from marine primary producers or allochthonous from terrestrial discharges (Nagata 2000, Rowe *et al.,* 2018) and its chemical composition, in which carbohydrates, amino acids and proteins are typically the most important components (Thornton 2014). The total marine DOM pool consists of 20−30 % of HMW compounds rich in carbohydrates, which frequently accumulate towards the end of the phytoplankton blooms (Biddanda & Benner 1997).

1.2.1 Processes of dissolved organic matter release

There are several processes involved in DOM production. One of them is sloppy feeding during grazing of micro- and mesozooplankton on phytoplankton and bacterioplankton (Sherr & Sherr 1996, Møller 2007). Among grazers, grazing by protists through the egestion of food vacuoles seems to be the dominant DOM release mechanism in oceanic waters, where small phytoplankton predominate (Nagata 2000). This process plays an important role not only in the dissolved organic carbon (DOC) flux, but also in the regeneration of other key components rich in iron, N and P (Johannes 1965). The total amount of DOC in the ocean has been estimated to be 662 Pg C (Hansell *et al.*, 2009). Another process involved in DOM release is viral lysis of phytoplankton, mostly during the decline of phytoplankton blooms, and

bacterioplankton cells (Gobler *et al.*, 1997, Suttle 2007). This released material, rich in N, P and nucleic acids, can be used by bacteria, which regenerate the inorganic nutrients through a bacterial-viral loop (Fuhrman 1999). Bacteria, through the use of ectoenzymes, can provoke the release DOM in phytoplankton cells or by further bacterial degradation of complex molecules (Azam & Malfatti 2007). Conversely, the degradation of labile DOC by bacteria can result in the production of more refractory compounds (Jiao *et al.*, 2010).

1.2.2 Dissolved organic matter released by phytoplankton organisms

The autochthonous production of DOM in marine systems is carried out by primary producers, as mentioned above. In the course of upwelling events and spring blooms, phytoplankton are the key players in the release of this DOM during their growth. Phytoplankton cells are microbes within a range of 0.6−2000 μm (most of them ≤ 200 μm) that perform photosynthesis. They incorporate dissolved inorganic carbon (DIC) in the form of carbon dioxide (CO_2) and bicarbonate (HCO_3^-) and inorganic nutrients, which are transformed into particulate organic carbon (POC) as phytoplankton biomass, using light as an energy source. Grazing can transfer this carbon biomass to higher trophic levels, producing and releasing smaller particles and DOM. This detritus material can be recycled within the euphotic zone or it can sink out from the surface, forming larger detrital aggregates, and the formation of these aggregates increases the sinking rate. For instance, diatoms form large blooms in response to inorganic nutrient inputs and sink rapidly, due to their silicate skeleton, after the depletion of inorganic nutrient(s) when the cell dies (Smetacek 1998, Ducklow *et al.* 2001). The aggregates can escape predation and decomposition in the water column and make it to the sea floor, where the carbon may be sequestered in the sediment for millions of years. Thus, phytoplankton play an important role in the carbon flux from surface to bottom waters in a process that is also known as the 'biological carbon pump' (Ducklow *et al.*, 2001). Moreover, phytoplankton are the main primary producers and form the basis of marine food webs, where their biomass can be transferred via grazing through a classical food chain, formed by phytoplankton-zooplankton-fish, or through the release of DOM and transfer to higher trophic levels through the microbial loop. The marine primary production (PP) has been estimated to 45–55 Pg C yr^{-1}, which corresponds to 40–50% of the global PP of the biosphere (Field *et al.,* 1998, Westberry *et al.*, 2008).

The share of the carbon fixation that is exuded or released as DOM by phytoplankton cells during photosynthesis can constitute up to 50% (Nagata 2000). There are two models that illustrate DOM release from living phytoplankton cells, as explained in detail by Thornton (2014): the leakage model, in which the cell releases LMW components constantly through the membrane as a passive process and following a concentration gradient (Bjørrisen 1988), and the overflow model, in which the cell excretes DOM polymers synthetized during photosynthesis as an active process to dissipate excess energy, *i.e.* under stress conditions of high irradiance, nutrient limitation or photorespiration (Fogg 1983, Baines & Pace 1991). Studies from natural waters have shown that there is an increased gradient of released DOM of phytoplankton origin from inshore to offshore waters (Williams 1990).

1.2.3 Key phytoplankton species in the release of dissolved organic matter

In general, diatoms have been the key organisms for studying phytoplankton production of DOM, due to their dominant role during phytoplankton blooms. Diatoms are capable of growing rapidly under replete inorganic nutrient conditions (r-strategy), and have, under such conditions, contributed highly to PP and to carbon export from surface waters to benthic ecosystems. For instance, *Skeletonema marinoi* and *Chaetoceros* spp. are two of the most commonly studied diatom species that contribute to these processes (Mague *et al.,* 1980, Myklestad 1995, Wetz & Wheeler 2007, Castillo *et al.,* 2010, Sarmento *et al.,* 2013). Overall, diatoms and dinoflagellates release DOM (Thorton 2014), mainly as carbohydrates (Myklestad 1974, Myklestad 1995), amino acids (Myklestad 2000, Castillo *et al.*, 2010, Sarmento *et al.*, 2013), fatty acids and lipids (Lombardi & Wangersky 1991, Parrish *et al.*, 1994). However, the release of

these components can be species-specific; *e.g.* the release of polysaccharide is higher in *Chaetoceros* spp. than in *S. marinoi* (Myklestad 1974). The production of DOC also differs between diatoms and dinoflagellates, being higher in *Prorocentrum* sp. than in *Skeletonema* sp. and *Chaetoceros* spp. (Castillo *et al.*, 2010). Unfortunately, relatively few studies have related the characteristics of DOM production to different taxonomic groups, and bloom-forming species have usually been studied for this purpose. The best-known examples include the prymnesiophyte *Phaeocystis* sp., a common blooming-forming species in the North Sea that produces high concentrations of carbohydrates and polysaccharide mucilage and releases DOC constantly during its growth, similar to the diatom *Skeletonema* sp. (Lancelot 1983, Biddanda & Benner 1997). Conversely, the cyanobacteria *Synechococcus* taxa, with high production of N-rich DOM, and the coccolithophore *Emiliania* sp. show higher DOC release during the stationary phase (Biddanda & Benner 1997) than the exponential growth phase.

1.3 Bacterioplankton and their role in marine ecosystems

Aerobic heterotrophic bacteria, as obligate osmotrophs, are microbes that consume DOM and inorganic nutrients via aerobic respiration and use oxygen as a terminal-electron acceptor (Kirchman 2000). Due to their small size and large surface-to-volume ratio, bacteria dominate the ocean in terms of abundance, diversity and metabolic activity compared with other living organisms (Williams 1981, Azam 1998). Moreover, they govern DOM mineralization, largely produced by phytoplankton (Pomeroy 1974, Azam *et al.,* 1983, Ducklow 1983, Cole *et al.*, 1988, Kirchman *et al.,* 1991), and can also take up, as well as release, inorganic nutrients (mainly N and P) during their metabolic processes (Kirchman 2000). The inorganic nutrients that have been remineralized by bacteria can be used again for PP (Azam et al. 1983, Buchan et al. 2014). This 'regenerated' production contrasts with new production, which is mainly carried out using nutrients from allochthonous sources, such as upwelling waters. In coastal upwelling systems, PP accounts for >10% of global new production (Chavez 1995). In the open ocean, regenerated production is typically more dominant than new production (Harrison 1993). During regenerative production, the DOM is incorporated and transformed into particulate organic matter (POM) conforming the bacterial biomass, which can be used by higher trophic levels via the microbial loop (Azam *et al.*, 1983, Cole *et al.*, 1988, Ducklow 2000). Marine heterotrophic bacteria also play an important role in the carbon flux, since they utilize the DOC, the most significant fraction of the DOM pool, as the main carbon source for their metabolic processes (Del Giorgio & Cole 1998). Thus, bacterioplankton are not just simple decomposers; they can recycle and transfer the organic matter and the inorganic nutrients within the ecosystem and up the trophic food web.

1.3.1 Bacterial metabolic processes

The estimation of bacterial metabolic rates is crucial to understanding and quantifying their role in marine ecosystems, *e.g.* their role in biogeochemical cycles of carbon, N and P. The carbon taken up by bacteria can be used for chemical energy production (ATP) by the oxidation of a substrate (catabolic processes = bacterial respiration, BR), and for biomass synthesis with an energy cost (anabolic processes = bacterial secondary production, BSP). The energy generated is also channelled into maintenance requirements, transport, growth and cell division.

From the estimation of the BSP and BR, two additional variables can be determined that describe bacterial carbon metabolism: the bacterial growth efficiency (BGE) and the bacterial carbon demand (BCD). BGE is dependent on the partitioning between the anabolic (*i.e.* BSP) and catabolic processes (*i.e.* BR) and is a measure of the fraction of carbon consumed that is incorporated into new biomass, whereas BCD determines the amount of carbon consumed by bacterioplankton (Del Giorgio & Cole 1998). High BGE and BCD values are associated with nutrient-rich and highly productive systems, such as coastal or estuarine areas, which also present high BSP and BR rates (Cole *et al.,* 1988, Ducklow & Carlson 1992,

Del Giorgio & Cole 1998). However, in oligotrophic systems and/or equatorial regions with high temperature, the BGE can decrease while the BCD remains high, and the bacterial community needs to adjust their metabolic functioning (Del Giorgio & Cole 1998, Hoppe *et al.*, 2002). Thus, under oligotrophic conditions with nutrient or organic carbon limitation, bacteria compete with phytoplankton for inorganic nutrient uptake (Kirchman 1994, Legendre & Rassoulzadegan 1995, Teeling *et al.*, 2012).

1.3.2 Bacterial production: origin of the dissolved organic matter flux through the microbial loop

The BSP estimates the synthesis of bacterial biomass as POM from dissolved organic and inorganic precursors, which in fact is the process that links the DOM pool with higher trophic levels through the microbial loop (Pomeroy 1974, Azam *et al.,* 1983). Thus, estimation of BSP is essential for understanding the role of bacterioplankton metabolism in the ecosystem (Cole *et al.*, 1988, Cole & Pace 1995).

In our studies, the BSP has been estimated by the simultaneous incorporation of two radio-labile precursors, DNA-[^{3}H] thymidine (TdR) and/or protein-^{3}H/^{14}C leucine (Leu), to yield the net rates of biomass synthesis (Fuhrman & Azam 1980, Kirchman *et al.*, 1985). The TdR incorporation method estimates the production of actively growing cells by tracing the nucleotide thymidine involved in DNA synthesis. In the case of the Leu method, the BSP can be used to estimate the protein synthesis by tracing the amino acid leucine, which is higher in fast-growing cells than in slow-growing cells. Both methods (TdR and Leu) result in similar estimation of BSP, even though their labels trace different processes (Kirchman 1992). The use of the dual approach of TdR and Leu incorporation allows the estimation of the Leu: TdR ratio, which gives insights into the growth rate and physiological status of the bacterial cell (Ducklow 2000). The ratio tends to increase under unfavourable conditions with low temperatures or chlorophyll *a* (Chl *a*), or when the cell shifts to new growth conditions (Shiah & Ducklow 1997, Camarena-Gómez *et al.*, 2018).

The drawback of these methods is the need to use of several conversion factors (CFs), to convert the incorporation rates of TdR and Leu in carbon-based BSP (Kirchman 1992). These can be empirically determined, but in practice most studies use already published, standard CFs. Besides, there are bacterial groups that do incorporate Leu and TdR at different rates: Betaproteobacteria incorporate Leu more efficiently than TdR, whereas Actinobacteria are more efficient at incorporating TdR (Pérez *et al.,* 2010). Moreover, the proportion of active cells within specific bacterial clades can vary, due to the external concentration of the macromolecule added (*i.e.* Leu). This variation in active cells incorporating TdR and Leu has also been observed along a salinity gradient (Cottrell & Kirchman 2003). Even though the variation in CFs can be used for calculating the BSP or the differences in labelled molecule uptake rates, the range of the BSP to PP ratio (BSP:PP) has been reported between 10% and 20% in different marine ecosystems (Ducklow 2000). The BSP:PP ratio is a useful estimator of the magnitude of the bacterial sink for organic carbon but caution must be taken in interpretation of the results (Ducklow *et al.*, 2002). To claim that the BSP is a certain fraction of the PP, the total PP (particulate + dissolved) must be estimated (Ducklow 2000).

1.3.3 Bacterial community structure and its environmental drivers

Alphaproteobacteria, represented by the SAR11 and *Roseobacter* clades*,* predominate in the majority of marine ecosystems and can be present during the entire year (Morris *et al.,* 2002, Alonso-Sáez *et al.*, 2007, Lindh *et al.*, 2015). SAR11 predominate in oligotrophic conditions that can be outcompeted by more opportunistic bacteria (Gómez-Consarnau *et al.*, 2012), whereas *Roseobacter* grows under high organic nutrient conditions (Gilbert *et al.*, 2012). There are environmental drivers, such as salinity, temperature, dissolved oxygen, inorganic nutrients and the phytoplankton-derived variables Chl *a*, PP, phytoplankton community composition and phytoplankton growth phase that, individually or in combination, can shape the bacterial community composition (BCC). Seasonality in temperate regions is another important factor that determines the bacterial community dynamics throughout the year. Its significance can be linked to environmental drivers such as temperature, salinity and phytoplankton-derived variables (Herlemann *et al.,* 2016, Bunse & Pinhassi 2017). Nevertheless, it has been observed

that SAR11 predominates in winter (Gilbert *et al.*, 2012, Herlemann *et al.*, 2016), Bacteroidetes and Gammaproteobacteria are associated with phytoplankton spring blooms and Verrucomicrobia, Actinobacteria and Planctomycetes appear later in the year (Andersson *et al.*, 2010, Herlemann *et al.*, 2011, Hugerth *et al.*, 2015, Lindh *et al.*, 2015).

Salinity also plays an important role in estuarine and brackish systems, with Alphaproteobacteria (saline SAR11) and Gammaproteobacteria predominating in high-salinity areas, whereas Actinobacteria, Betaproteobacteria and also the fresh water lineage of SAR11, predominate in freshwater areas (Bouvier & del Giorgio 2002, Logares *et al.*, 2009, Herlemann *et al.*, 2011, Hugerth *et al.,* 2015, Herlemann *et al.*, 2016, Kirchman *et al.,* 2017). Dissolved oxygen appears to be an important driver of the bacterial community in systems with steep oxygen gradients, such as in the eastern tropical South Pacific Oxygen Minimum Zone (OMZ) off northern Chile (Stevens & Ulloa 2008, Wright *et al.*, 2012, Aldunate *et al.*, 2018). Temperature can also modulate the bacterial activity and affect BCC (Fuhrman *et al.*, 2008, Hoppe *et al.*, 2008, von Scheibner *et al.*, 2014). For instance, Andersson *et al.* (2010) observed that temperature and phosphate were the factors shaping the bacterial community in the Baltic Sea. In addition, Pearman *et al.*, (2016) observed that the BCC was affected by nutrient input (ammonium) and likely by the phytoplankton bloom phase, but was not affected by the phytoplankton community composition during a mesocosm experiment.

Regarding the phytoplankton community composition and/or growth phase, differences in the quality and quantity of the DOM pool can shape the metabolism, structure and dynamics of the bacterioplankton (Cottrell & Kirchman 2000, Sarmento & Gasol 2012, Sarmento *et al.,* 2013). BSP rates can differ between diatoms and dinoflagellates (Lekunberri *et al.*, 2012, Camarena-Gómez *et al.*, 2018). Thus, it has been suggested that the quality of the DOM related to DOC lability plays an important role in bacterioplankton metabolism (Apple & Del Giorgio 2007). In addition, several patterns regarding phytoplankton-bacterioplankton occurrence have been described. For instance, various diatom taxa such as *Chaetoceros* spp., *Skeletonema* sp. and *Thalassiosira* sp. favour the occurrence of Bacteroidetes and the *Roseobacter* clade (Riemann *et al.*, 2000, Pinhassi *et al.,* 2004, Grossart *et al.*, 2005). There are bacterial groups that are commonly observed during phytoplankton blooms (Teira *et al.*, 2008, Teeling *et al.*, 2012, Buchan *et al.*, 2014) and upwelling systems (Stevens & Ulloa 2008, Aldunate *et al.*, 2018). Gammaproteobacteria, frequently called pulse populations due to the briefness of their abundance peaks, can also appear during phytoplankton blooms (Teeling *et al.*, 2012, Camarena-Gómez *et al.,* 2018), *e.g.* the genus *Alteromonas* (Sarmento & Gasol 2012, Sarmento *et al.*, 2013). They can also appear due to other changes in the environment, such as in nutrient availability. Teira and collaborators observed that Gammaproteobacteria positively correlated with inorganic nutrients (nitrate, nitrite and silica) and negatively with DOC concentration, whereas Bacteroidetes correlated positively with percentage of extracellular release (PER) and high DOC concentrations (Teira *et al.*, 2008). This was likely due to their different preferences for HMW (Bacteroidetes) or LMW (Gammaproteobacteria) organic matter (Cottrell & Kirchman 2000, Pinhassi & Berman 2003). *Alteromonadales* (Gammaproteobacteria) and *Flavobacteriia* (Bacteroidetes) can also degrade transparent exopolymer particles (TEPs), which are polysaccharides rich in organic sulphate compounds (Passow 2002), since they have exoenzymes that can break-down these polymers (Teeling *et al.*, 2012, Buchan *et al.*, 2014, Taylor *et al.*, 2014). The *Roseobacter* clade increased with the TEP concentrations, but this clade likely used a more available substrate originating from the previous degradation of TEPs carried out by *Alteromonadales* and/ or Flavobacteriia (Taylor & Cunliffe 2017). The *Roseobacter* clade is known to secrete exoenzymes that degrade *Synechococcus*-derived polymers (Christie-Oleza *et al.,* 2015). Thus, it has been suggested that *Alteromonadales*, *Flavobacteriia* and *Roseobacter* function as the 'master recyclers' of diatom-derived polysaccharides (Taylor & Cunliffe 2017, Mühlenbruch *et al,.* 2018). Flavobacteriia (Cytophagia-Flavobacter clade) are also known to consume chitin, N-acetylglucosamine (NAG) and proteins (Cottrell & Kirchman 2000, Eckert *et al.,* 2012). Chitin is a polymer of NAG found in the cell wall of diatoms (Durkin *et al.*, 2009). Flavobacteriia has also been observed during dinoflagellate blooms (Fandino *et al.*, 2001, Camarena-Gómez *et al.*, 2018), suggesting that they can use dinoflagellate-derived DOM.

1.4 The microbial loop : the link between bacterioplankton and higher trophic levels

The paradigm of the microbial loop, or microbial food chain, was already suggested by Keys *et al.,* (1935) and later by Pomeroy (1974) and Larsson & Hagström (1982). Azam et al. named it the 'microbial loop' in 1983, a term that has been widely adapted for the microbial food chain. The microbial loop is a trophic pathway within marine microbes that transfer DOM, largely released by phytoplankton, to higher trophic levels through the incorporation of this DOM as bacterial biomass. The integration of this process in the marine food webs caused a revolution in biological oceanography, since it introduced the important role of bacterioplankton in marine ecosystems. Later, the role of viruses in the carbon cycle via the bacterial-viral loop was included in the microbial loop. This bacterial-viral loop also plays an important role in the microbial loop via viral-lysis of bacteria that cause the release of the bacterial contents and potentially increases BSP and BR with the consequent regeneration of inorganic nutrients (Fuhrman 1999). It is now evident that the classical food chains are too simple to explain the structure and dynamics of the marine plankton communities (Fenchel 2008).

With the discovery of the role of the bacterioplankton and the introduction of the microbial loop paradigm, a debate about the 'link or sink' property of the microbial loop emerged (Pomeroy 1974); it is named 'sink' where the carbon is mostly respired and lost from the system, or 'link' where the carbon is channelled to higher trophic levels. Ducklow (1983) concluded that the microbial loop is a sink for organic carbon, which is supported by the low BGE values (< 40 %) generally found in marine ecosystems. Thus, there is more carbon respired in the microbial loop than carbon forming new biomass being available for higher trophic levels. This was attributed to the introduction of additional trophic levels within the food web before the carbon reaches the zooplankton level (Williams 2000). More trophic levels cause a loss of energy efficiency or C transfer due to the respiration of organic C and release of CO_2 for each trophic level (Berglund *et al.*, 2007, Kirchman 2012). Nevertheless, the microbial loop is still important as a link between the primary producers and upper trophic levels of the marine food web, since it provides supplemental organic material to metazoans (Williams 1981).

1.5 Effects of global change on phytoplankton communities

Currently, the entire Earth system is facing large-scale changes related to human activity and energy use that are causing unprecedented effects on biogeochemical cycles. Global warming, induced by human release of CO_2, is without doubt the most alarming pressure affecting marine ecosystems worldwide (Petchey *et al.,* 1999, Halpern *et al.*, 2008, Duarte 2014). There has been a decrease in the standing stock of phytoplankton over the last century, caused by increasing surface temperature (Boyce *et al.,* 2010). The phytoplankton community composition is also changing, as long-term data sets have revealed, *e.g.* in the North Atlantic (Leterme *et al.*, 2005), North Sea (Hinder *et al.*, 2012, Capuzzo *et al.,* 2018), Mediterranean Sea, (Mercado *et al.*, 2007) and Baltic Sea (Klais *et al.*, 2011, Wasmund *et al.*, 2011). The onset of the phytoplankton blooms has been affected in temperate coastal systems, either starting earlier (Winder & Sommer 2012, Groetsch *et al.*, 2016, Kahru *et al.*, 2016) or being delayed (Nixon *et al.*, 2009). But there are further drivers/pressures that can synergistically affect the phytoplankton response, making it difficult to predict the effect of global change. Among them, enhancement of stratification and deoxygenation (Keeling & Garcia 2002), ocean acidification (Turner *et al.*, 2018), eutrophication (Rabalais *et al.*, 2009) or the increase in ultraviolet radiation (Häder *et al.*, 2007) are other pressures that interact with the increased temperature, affecting phytoplankton metabolism, composition and structure.

1.5.1 Changes in the Baltic Sea ecosystem

The Baltic Sea is one of the ecosystems studied here, and it is suffering from eutrophication, mostly in the Gulf of Finland (GoF), due to the large number of people in the catchment area of this basin (Fleming & Kaitala 2006, HELCOM 2009). In addition, global warming has been projected to increase the surface temperature and wind stress and to decrease the ice cover (Meier *et al.*, 2011b, Christensen *et al.,* 2015).

This will cause more precipitation with the consequent increase in fresh-water runoff (Meier 2006) and discharges of terrestrial organic pollutants (Fleming-Lehtinen *et al.*, 2015, Tamelander *et al.*, 2017) that will dilute the salinity in the northern sub-basins (Andersson *et al.*, 2015). Higher acidification and expansion of hypoxic or anoxic shallows are also projected for the future Baltic Sea (Meier *et al.*, 2011a, Turner *et al.*, 2018).

Eutrophication has led to changes in the inorganic nutrient regime, due to the release of P from anoxic sediments in some sub-basins of the Baltic Sea, such as the GoF and the Baltic Proper (BP), which veil the decrease in nutrient loading observed since the 1990s (Pitkänen *et al.*, 2001, Stigebrandt *et al.*, 2014). The nitrate and phosphate pools have changed in recent decades towards the depletion of P in the Bay of Bothnia (BoB) and depletion of N in the BP and GoF after the spring phytoplankton bloom (Andersson et al. 1996, Tamminen and Andersen 2007). The difference in N and P pools constituted a feature of these subbasins, frequently defined as N-limited or P-limited regions. The eutrophication pressure observed in the N-limited subbasins will be aggravated, due to the increase in allochthonous nutrient loading, the expansion of the anoxic-hypoxic bottoms and the continued release of P from the sediment (Meier *et al.,* 2011a, Meier *et al.,* 2012) benefitting N-fixing cyanobacteria (Vahtera *et al*., 2007).

The tendency for the dissolved inorganic nitrogen to dissolved inorganic phosphorus (DIN:DIP) ratios to decrease from the northern to the southern Baltic Sea will differentially affect the PP in the various subbasins of the Baltic Sea. For instance, in the northern Gulf of Bothnia, the synergistic effect of P limitation and increased DOM from terrestrial origin can restrict phytoplankton productivity and favour the development of heterotrophic bacteria that can outcompete phytoplankton in N uptake (Meunier *et al.*, 2017, Rowe *et al.*, 2018). Thus, more heterotrophic organisms are expected to occur, altering the structure of the food web towards a heterotrophic bacteria-based food web (Wikner &Andersson 2012, Andersson *et al.,* 2015). In contrast, PP is expected to increase in the GoF and the BP, since they both are affected by continuous nutrient loads (Meier *et al.*, 2011b). Global warming in the Baltic Sea is also affecting the spring phytoplankton bloom dynamics. The spring bloom in the GoF and northern BP is having an earlier onset (February-March) and lower biomass peak than previously (Tamelander & Heiskanen 2004, Fleming & Kaitala 2006, Groetsch *et al.,* 2016). In addition, earlier developments of cyanobacteria and zooplankton summer blooms, with compositional changes in the zooplankton community, have been observed (Suikkanen *et al.,* 2013, Karhu &Elmgren 2014). These changes could lead to a better match between the phyto- and zooplankton blooms during spring and an increase in the grazing pressure (Aberle *et al*., 2012, Tamelander *et al*., 2017). Consequently, the vertical flux of organic material to the sediment may decrease while the energy transfer to higher trophic levels may increase (Lignell *et al.*, 1993, Aberle *et al.*, 2012).

A shift in the phytoplankton community composition towards higher dinoflagellate abundance and the co-occurrence of diatoms and dinoflagellates in the early spring bloom have been observed in parts of the Baltic Sea (Klais *et al.*, 2011, Wasmund *et al.*, 2011). These changes were likely related to the increase in temperature and light availability, due to the ice-free winters (Klais *et al.*, 2013) and can have important consequences for the biogeochemistry of the basin. Heiskanen (1998) demonstrated that both groups differ in their sedimentation patterns (Fig. 1). Diatoms settle quickly to the sea floor, due to their silica exoskeleton. They transport more organic material with a higher C:N:P ratio than dinoflagellates (Spilling *et al.*, 2014), which either break before reaching the sediment or settle as dormant resting cysts. The dinoflagellate cysts that sink out of the water column do not degrade easily, and thus this material does not significantly contribute to the hypoxic conditions at the sea floor, *e.g.* by reducing the flux of P out of the sediment (Spilling & Lindström 2008). The results demonstrate that the phytoplankton community composition has clear implications for the benthic ecosystem, since the quantity and quality of the settling material affects carbon and nutrient fluxes (Spilling *et al*., 2018). Additionally, the quality and quantity of the DOM released by diatoms and dinoflagellates may variously affect the bacterioplankton responses. To our knowledge, the consequences of these differences on BCC and BSP are poorly known and form one of the main objectives of this PhD thesis.

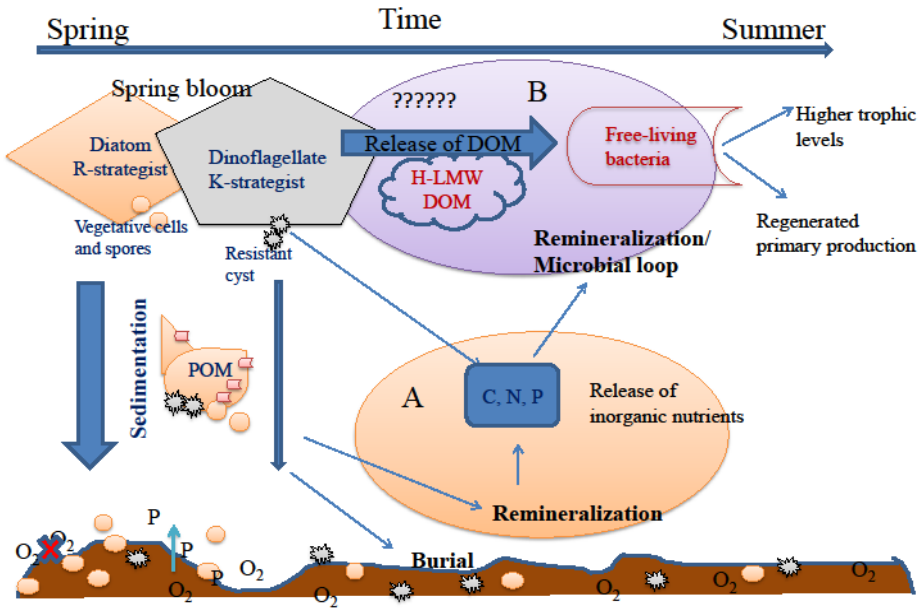


Figure 1. A conceptual model of the fate of organic material produced during the spring bloom in the Baltic Sea. Long-term monitoring data suggest a shift in the spring community from diatom to dinoflagellate dominance (Klais *et al.* 2011). During a diatom-dominated spring bloom, pathway A, with higher transport of organic material to the sediment, predominates. Pathway B takes over during dinoflagellate dominance and cyst formation. Lack of information about DOM release from diatoms and dinoflagellates rendered us unable to decipher its effects on bacterioplankton responses, and therefore, on the entire microbial loop.

1.5.2 Changes in the Humboldt Current System

The other ecosystem studied in this thesis was the Humboldt Current System (HCS), in northern-central Chile. Global warming is causing an increase in surface-water temperature and stratification with consequent decrease in gas solubility and dissolved oxygen concentration in the HCS (Keeling and Garcia 2002). In addition, the remarkable productivity of this region causes intense organic matter degradation by microbial respiration (Daneri *et al.*, 2000). The naturally occurring OMZ has expanded in recent decades (Stramma *et al.,* 2008). Nearshore, the oxycline (upper limit of the OMZ) is located at a depth of 50 m and has been detected as close to the surface as 10 m after a strong El Niño event (Montecino et al. 2013). Under low dissolved oxygen conditions and high microbial rates, both the biological pump and the biogeochemical cycles, *i.e.* mainly N and P, can be significantly affected (Fig. 2). In the case of the N cycle, denitrification and anaerobic ammonium oxidation (Anammox) remove the inorganic N (nitrate NO_3^-, nitite NO_2^-, and ammonium NH_4^+ ions) with the production of N_2 (Codispoti *et al.*, 2001, Thamdrup *et al.,* 2006). Regarding the P cycle, P is bound to Fe (Fe^{3+}) in oxidized sediment, but is released under anoxic conditions. Thus, the upwelling waters in the HCS can result in higher P and lower N concentrations, reducing the inorganic N:P ratio, due to the expansion of the OMZ in sub-surface waters (Codispoti *et al.*, 2005). This reduction in the N:P ratio can affect the trophic energy transfer in the pelagic food web and also the phytoplankton community composition, *i.e.* promoting a decrease in diatoms abundance (Franz *et al.*, 2012, Hauss *et al.*, 2012). The occurrence of diatom and dinoflagellate blooms during upwelling events is also well known in this system (Wieters *et al.*, 2003, Anabalón *et al.*, 2016) and may be affected by processes similar to those explained in the previous section regarding the effects of global change in the Baltic Sea.

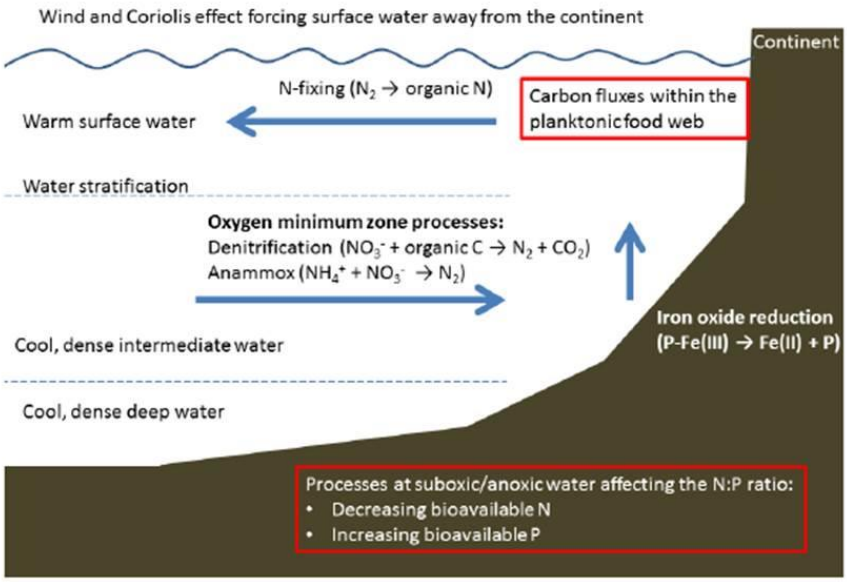


Figure 2. A conceptual model of the fate of inorganic nutrients (N, P) during upwelling events in the Humboldt Current System off Chile. The expansion of the Oxygen Minimum Zone enhances anoxic processes such as denitrification and anammox decreasing the N and increasing P availability. This type of water characterized by low N:P ratios may reach the surface by upwelling and affect the phytoplankton community composition.

2 OBJECTIVE OF THE THESIS

The general objective of this thesis was to determine the effects of diatom- and dinoflagellate-dominated communities on the associated bacterial structure (functioning and diversity) during phytoplankton blooms. This was achieved by combining experimental (Chapters I, III, from here on I and III) and field work (Chapter II, from here on II) to follow the bacterial response in different diatom- and dinoflagellate-dominated communities. The experiments were conducted in the GoF (I) and the HCS off Chile (III), and the field campaigns were conducted throughout the Baltic Sea (II) during and after the phytoplankton spring bloom in four consecutive years. In the Baltic Sea diatom- and dinoflagellate-dominated communities co-occur during spring, while in the HCS (III) diatom- and dinoflagellate-dominated communities are also commonly observed in different upwelling locations. Thus, these systems share a common feature: the occurrence of diatom and dinoflagellate blooms, besides being N-limited systems (except the BoB), although they differ in terms of their physico-chemical features and environmental pressures on phytoplankton. This allowed me to test whether there were similar patterns in bacterial community structure in the various systems (Baltic Sea and Humboldt Current), depending on the distinct phytoplankton groups. Further studies were conducted in the Baltic Sea to estimate the changes in bacterial functioning and diversity by analysing BSP (I, II), BR (II), bacterial abundance (BA) (I, II) and bacterial diversity (II) during the various phytoplankton blooms. In addition, further environmental factors (physicochemical and biological) were analysed to establish their relevance in shaping the bacterial community structure (II).

3 MATERIALS AND METHODS

3.1 Study area

The background research for this thesis was conducted in two ecosystems: in the Baltic Sea, more specifically in the coastal area of the GoF, and in the HCS off Chile (Fig. 3).

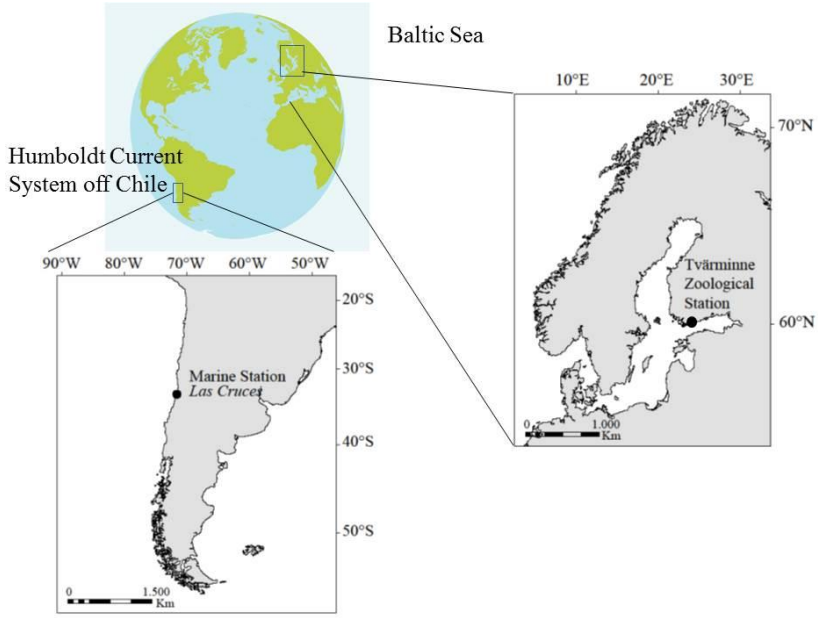


Figure 3. Map of the study sites in the Baltic Sea (I, II) and the Humboldt Current System off Chile (III). Map of the Earth, indicating both sampling sites. The dot symbols indicate the location of the marine stations where the experiments were conducted.

3.1.1 Baltic Sea (I, III)

The Baltic Sea is a stratified and semi-enclosed catchment area that covers 392978 km^2. It is divided into different subbasins with their own hydrodynamic and physicochemical features (Fig. 4). The water retention time in the central Baltic Sea is between 3 and 30 years (Kullenberg 1982). The system is characterized by its shallowness, with an average depth of 54 m. Another feature of this system is the strong salinity gradient ranging from 2 at the norther BoB, largely influenced by the fresh-water run-off, to 30 at the bottom layers of the Danish Straits. This salinity gradient makes this one of the largest brackish ecosystems worldwide, which is the main feature defining the stratification of the water column. Seasonality, based on temperature changes throughout the year, is another feature of the Baltic Sea. The surface-water temperature is minima (freezing point) in winter, but increases with depth to the temperature of maximum water density, forming an inverse thermocline and maximum in summer. In spring, the ice retreats, the surface layer warms up and the salinity decreases to its minimum, due to the enhancement of river discharge. In summer, the presence of a thermocline prevents vertical mixing and causes the stratification of the water column. There is also a gradient from north to south in the inorganic and organic nutrient concentrations. One of the most particular characteristics of the Baltic Sea is the high DOM concentration. The DOC concentration in the Baltic Sea is 3−6 times higher than in other sea areas, *e.g.* the Atlantic Ocean, ranging from 250 to 500 µM C (Hoikkala *et al.*, 2015).

The GoF, one of the subbasins where the GoF experiments were conducted, is located in the northeastern part of the Baltic Sea and is connected with the BP. This subbasin is influenced by the Neva River discharges, causing a horizontal salinity gradient from 4 in the eastern end of the basin to 6 in the western GoF.

3.1.1.1 Phytoplankton bloom in the Baltic Sea

A phytoplankton spring bloom, dominated by diatoms and dinoflagellates, develops each year in the Baltic Sea (Wasmund *et al.,* 1998, Tamelander & Heiskanen 2004). The representative species of diatoms include *Achnanthes taeniata* and *Melosira arctica*, associated with the winter ice-forming communities; *Chaetoceros wighamii*, *Thalassiosira baltica*, *T. levanderi* and *S. marinoi*, which typically increase in relative abundance later in the bloom (Spilling 2007). In the case of dinoflagellates, a complex is identified in the Baltic Sea formed by three different species: *Biecheleria baltica*, *Gymnodinium corollarium* and *Apocalathium malmogiense,* which historically was identified as *Scrippsiella hangoei*. *B. baltica* is mainly found in the GoF (Olli & Trunov 2010) and *G. corollarium*, mainly in the BP (Sundström *et al.*, 2009) and are the species that clearly contribute most to the dinoflagellate blooms. Both, together with the chain-forming *Peridiniella catenata,* are some of the most frequent dinoflagellate species encountered together with diatoms during the spring bloom.

The spring bloom in the Baltic Sea is the most important phase of the phytoplankton annual cycle in terms of new production and carbon fixation; approximately 40−60 % of total PP occurs during a few weeks (Lignell *et al.*, 1993, Heiskanen 1998). But the occurrence of the spring bloom differs by subbasin, starting in the southernmost Baltic Sea in February/March, reaching GoF in April and travels northwards into the Gulf of Bothnia in May (Kahru & Nômmann 1990). The increase in light and inorganic nutrient availability, caused by the retreating ice cover and the formation of a temporal halocline, promotes the growth of the spring bloom and increase in net PP (Stipa 2004). It peaks at the time when inorganic nutrients have been depleted; N-limitation prevails in most of the Baltic Sea, with the exception of the BoB, which is P-limited (Andersson *et al.*, 1996, Hagström *et al.,* 2001, Tamminen & Andersen 2007), causing the collapse of the bloom. The decline phase is characterized by rapid sinking of the phytoplankton cells (Heiskanen 1998), followed by the postbloom phase, with lower phytoplankton biomass mainly driven by recycled production (Hagström *et al.,* 2001). This organic material settles, due to the mismatch between the phytoplankton and mesozooplankton occurrence and the scarcity of grazing pressure (Lignell *et al.,* 1993, Wasmund & Uhlig 2003, Höglander *et al.,* 2004).

The spring bloom also differs in intensity, being the highest in terms of Chl *a* in the GoF (Fleming and Kaitala 2006). This high intensity is reflected in the PP values (> 30 g C m^{-2}) observed in this subbasin during the spring bloom, and the corresponding bacterial thymidine production (BPT: 1.75 g C m^{-2}), which constitutes 6 % of the total PP (Lignell 1990). Thus, the BCD in this system seems to be supported by autochthonous DOM (Hagström *et al*., 2001).

3.1.2 Humboldt Current System (II)

Based on the physical characteristics of the Chilean coast, this region is divided into four zones (Figueroa 2002). The northern region (> 32°S) is characterized by a narrow shelf (<10 km) with low wind force and freshwater inflow. The central region (from 32°S to 36°S), has a wider shelf and the wind is more intense. From 36°S to 42°S, there is more freshwater inflow and wind forcing. At latitudes > 42°S, there are more poleward winds and the shelf is characterized by the presence of fjords. The central region is also characterized by the influence of the Maipo River (33.61°S) and the San Antonio Submarine Canyon (Wieters *et al.*, 2003, Narváez *et al.*, 2004, Piñones *et al.*, 2005).

The HCS belongs to four major Eastern Boundary Upwelling Systems (EBUSs), and these highly productive areas have some of the highest fish captures worldwide (Montecino & Lange 2009). This system originates from the deviation of the West Wind Drift (WWD) Current at ~ 42°S, and continues towards the north along the western shore of the Chilean and Peruvian coast. The action of equatorward wind forcing with periods of relaxation along the shore and the Ekman transport offshore, with the

influence of the Southeast Pacific Subtropical Anticyclone, brings up sub-surface nutrient-rich waters by upwelling events that support the high PP of this ecosystem (Montecino *et al.,* 2006, Thiel *et al.,* 2007). This water mass is the Equatorial Subsurface Water (ESSW), which is characterized by its low oxygen and high inorganic nutrient concentrations (Silva and Neshyba 1979). Another feature of the HCS in Chile is the latitudinal and seasonal temperature and salinity changes, being warmer and saltier in northern Chile than in the southern region (Montecino et al. 2013). There are also seasonal changes in the wind patterns, being the winds from the west more intense in spring and summer and absent in winter (Daneri *et al.,* 2000, Narváez *et al.*, 2004).

3.1.2.1 Phytoplankton blooms in the upwelling Humboldt Current System

The complex topography and meteorological changes make the HCS highly heterogeneous, affecting the range of Chl *a*, phytoplankton bloom dynamics and PP during the austral upwelling season (Aguilera *et al.*, 2018). In addition, the area is affected by the ENSO, which introduces a strong inter-annual variation warming up the coastal waters and consequently decreasing productivity during El Niño. Iron availability is also known to limit PP in the central to southern areas (Martin & Gordon 1988, Moore *et al.,* 2013). For instance, northern Chile (21−23°S) has permanently high PP values during the year, although high Chl *a* concentrations peak in winter and spring, due to seasonal phytoplankton blooms (Daneri *et al.*, 2000, Thomas *et al.,* 2001). Here, we can find long chain-forming diatoms such as *Chaetoceros* spp. and *Thalassiosira* spp. (Iriarte & González 2004). In the central to southern areas the upwelling mainly occurs in spring to summer with the Chl *a* peaking in summer (Thomas *et al.,* 2001). This high PP supports high BSP rates in this region (Troncoso *et al.*, 2003, Montero *et al.,* 2007). Thus, the carbon flux through the microbial loop is important in this upwelling region, in addition to the classical herbivorous food web, which has been discussed (Vargas *et al.*, 2007).

Our experiment was carried out on the central Chilean coast (33°S), between two well-known upwelling areas: Punta Curaumilla (33.1°S) in the north and Punta Toro (33.8°S) in the south (Wieters *et al.*, 2003, Narváez *et al.*, 2004, Piñones *et al.*, 2005). The three sampling sites of our study coincided with the locations described in Wieters *et al*., 2003: Location 'North' (33.12°S), close to Punta Curaumilla, a recurrent upwelling area; Location 'Central' (33.21°S); Location 'South' (33.35°S), close to Punta Toro and with the influence of the Maipo River, which likely plays a significant role in the productivity of this region. This area is a nonactive upwelling area, but it is considered as an upwelling trap, since the warmer waters of this area are surrounded by cold-water filaments (Hormazabal *et al.*, 2001, Wieters *et al.,* 2003).

3.2 Summary of the methods (I−III)

The methods and variables measured in this study are summarized in Table 1 and described in detail in articles I, II and III.

	Variable	Method	Reference	I	II	III
Bacteria	Net heterotrophic production	^{3}H-Thymidine and ^{14}C-Leucine incorporation method				
		Filtration	Furhman & Azam 1980, 1982	2	-	-
		Centrifugation	Smith & Azam 1992	**1**	**1**, 2	-
	Abundance	Flow cytometer	Gasol & del Giorgio 2000	2	2	-
	Respiration	Tritiation method (RQ 1)	del Giorgio *et al.* 1997 Koch et al. 2007	-	**1***	-
	Community composition	DNA extraction	DNA isolation kit	**1**	**1**	**1**
	Sequencing	Illumina MiSeq		3	3	3
Phytoplankton	Primary gross production	^{14}C method	Nielsen 1952; Gargas 1975	2	2	-
	Net percentage of extracellular release (PER)	Filtered DO^{14}C through <0.2 µm after 24h incubation	Morán & Estrada 2002	-	2	-

	Chlorophyll *a*	Filtration, ethanol extraction and spectrophotometric detection	Jespersen & Christoffersen 1987	4	5	5
	Community composition	Inverted light microscopy	Utermöhl 1958	2	2	2
	Carbon biomass	Conversion of cell number to carbon	Olenina *et al.* 2006, Menden-Deuer & Lessard 2000	2	2	-
	Diatom and dinoflagellate carbon biomass*		Wasdmund *et al*. 2017	2	2	2*
	Bloom phase definition			**1**, 2	-	**1**, 2
Nano/micro-Zooplankton	Carbon biomass	Conversion of cell number to carbon	auf dem Venne 1994, Banchetti *et al.* 1989 Olenina et al. 2006	2	2	2
	Community composition	Inverted light microscopy	Utermöhl 1958	2	2	2
Nutrients	Inorganic nutrients (NO_2+NO_3-N, NH_4-N, PO_4-P, DSi)	Standard colorimetric method	Grasshoff *et al.* 1983	4	5	2
	Particulate organic nutrients (POC, PON, POP, BSi)	Filtration	Salonen *et al.* 1979, Solorzano & Sharp 1980, Krausse *et al.* 1983	3	5	6
	Dissolved organic nutrients (DOC, DON)	Filtration	Benner *et al*. 1993	3	5	6
Environmental Variables	Temperature, salinity, depth, dissolved oxygen, Chl *a* fluorescence	Conductivity-temperature-depth sensor (CTD)		-	5	2
	Temperature			2	-	2
	Salinity			2	-	2
Bioinformatics	Post-processing sequencing data					
	Primer removal	Cutadapt	Martin 2011	2	2	2
	Merged reads	PEAR software	Zhang *et al.* 2014	2	2	2
	Chimera checking		Edgar *et al.* 2011			
		UPARSE pipeline	Edgar 2013	2	-	2
		UPARSE pipeline	Logares R 2017	-	**1**, 2	-
	Taxonomic classification 97 %	Mothur	Quast *et al.* 2013 Schloss *et al.* 2009	2	-	2
	Taxonomic classification 99 %	UPARSE pipeline	Logares R 2017	-	**1**, 2	-
	Normalization	metagenome Seq R Development Core Team 2011	Paulson *et al.* 2013	1	-	
	Rarefaction	*rrarefy* (RStudio)	Gotelli & Cowell 2001	-	-	**1**
Multivariable analysis	NMDS, RDS forward selection, Mantel test, Spearman correlations	RStudio	RStudio Team 2015	**1**	**1**	**1**
Statistics	One- or two-way ANOVA, Tukey's b-test			**1**, 2	-	**1**, 2
	Kruskal Wallis, Wilconxon test			**-**	1	**-**
	PERMANOVA	Primer/Permanova	Anderson *et al.* 2008	**1**	**-**	**1**

Table 1. Summary of the methods used in articles I−III. The numbers denote the staff in charge of the analysis: 1 = MT Camarena Gómez; 2 = other authors; 3−6 = external service at Institute of Biotechnology, University of Helsinki, Finland (3); Tvärminne Zoological Station, University of Helsinki (4); Marine Research Centre, Finnish Environment Institute (5); Universidad Católica de Valparaíso, Chile (6). *Results presented in the thesis and not included in the articles.

3.3 Data collection

To carry out this PhD thesis we conducted three experimental studies and four cruises; two experiments were conducted at the Tvärminne Zoological Station, University of Helsinki, located in the GoF subbasin, and one at the Marine Station Las Cruces located at the HCS off Chile (Fig. 3). The field study was conducted along the Baltic Sea covering the subbasins GoF, Åland Sea (ÅS), Archipelago Sea (ArS), BP, Bothnian Sea (BS) and BoB.

3.3.1 Experimental study in the Gulf of Finland (I)

The experiments were carried out in winter 2012 (March) and 2013 (February) at the Tvärminne Zoological Station with water collected from the ice edge at Storfjärden (59°50'N; 23°15'E), western GoF (GoF experiment). The water was collected at a depth of 20 m to avoid any freshwater influence from a nearby river and was characterized by low temperature (< 1°C), low salinity (~ 5), high inorganic nutrient concentration (*e.g.* nitrate > 80 µg L^{-1}) and low phytoplankton carbon biomass at the sampling time. In both experiments, the water collected was filtered through a 200 µm mesh and transferred to clean (acid-washed) carboys. Twenty litre polycarbonate carboys were set in a climate room at 4 °C and 70 µmol photons m^{-2} s^{-1} irradiance under a 12:12 h light:dark cycle (Fig. 4). This temperature was maintained during the phytoplankton bloom development. After the inorganic nutrient depletion, the temperature was increased to 10 °C to simulate the spring natural environmental conditions of warming the surface water and was set as the bacterial bloom phase.

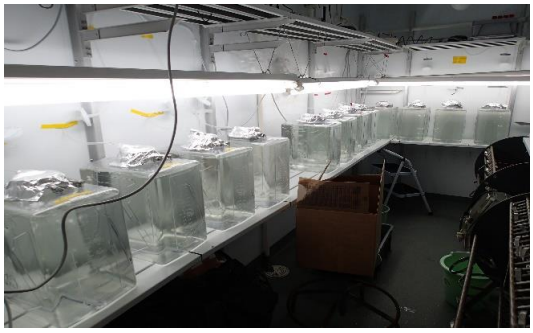


Figure 4. Experimental set up of the Gulf of Finland experiment in a climate room at the Tvärminne Zoological Station.

The effect of the phytoplankton community composition on bacterioplankton metabolism and composition was followed after the addition of diatom and dinoflagellate monocultures to the natural community. The phytoplankton addition was aimed to ensure the shift of the initial natural phytoplankton community towards diatom or dinoflagellate dominance. Thus, the natural communities constituted the controls (CONTR1 and CONTR2) in both years. In 2012, there were two treatments: diatom treatment (DIATOM) with the addition of the species *T. baltica* and *Chaetoceros wighamii,* and dinoflagellate treatment (DINOF) with the addition of *A. malmogiense*, *G. corollarium*, and *B. baltica*, referred to as dinoflagellate complex. In 2013, there were three treatments that were named by the initials of the monocultures added: *A. taeniata* (AT) and *T. baltica* (TB) as diatom-dominated treatments; *B. baltica* (BB) as the dinoflagellate-dominated treatment. The biomass addition of the monocultures, based on Chl *a* concentrations, was 0.7 µg L^{-1} in the DIATOM and 0.4 µg L^{-1} in the DINOF treatments in 2012 and 0.2−0.3 µg L^{-1} in all the treatments in 2013 (Table 2). The additions were 1.4 % and 0.8 % of the Chl *a* maximum in the DIATOM and DINOF treatment, respectively, and 0.2 % of the total volume at the time of the addition. The additions in the AT, TB and BB treatments in 2013 represented the 0.06 % of the Chl *a* maximum and the 0.05 % of the total volume at the time of the addition. There were three replicates per

treatment, thus 9 carboys in 2012 and 12 carboys in 2013. The sampling was carried out 1−3 times per week depending on the variable. Results showing the plankton carbon biomass are from the starting day and the Chl *a* peak. Results showing the bacterioplankton community composition are from the starting day, the Chl *a* peak and the end of the experiment in 2012 and at the bacterial production peak in 2013.

3.3.2 Field study in the Baltic Sea (II)

Four cruises were conducted during and after the spring phytoplankton bloom in April 2013 and 2016 and in May 2014 and 2015 along the Baltic Sea (Fig. 5). In total, 127 samples were collected at a depth of 3 m on board the vessel Aranda from seven different sub-basins: GoF, ÅS and BP in the cruises carried out in April; GoF, ArS and north BP in 2014 with the addition of BS, Kvarken and BoB in 2015. The water was collected using a sampling rosette equipped with 5-L Niskin bottles or from the flow-through system on-board (four stations in 2013). The unfiltered water was placed in a clean polycarbonate bottle for further analysis. All the bottles were rinsed with the sampling water at the time of the collection.

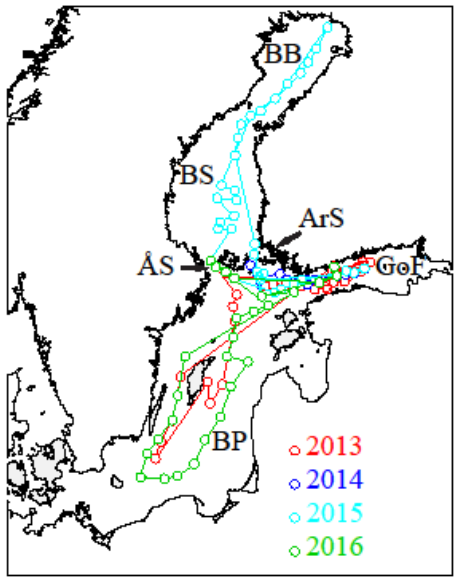


Figure 5. Map with the routes of the various cruises along the Baltic Sea. Note that different sub-basins were covered in each cruise. The colour-code indicates the cruises in each year.

3.3.3 Experimental study in the Humboldt Current System off Chile

The experiment in the HCS off Chile (HCS experiment) was carried out during the austral summer (March) in 2014. The water was collected at three study sites (North, Central and South) that differed in the frequency of the upwelling events and the initial phytoplankton community composition: 'North' and 'Central' are frequently affected by upwelling events and dominated by diatoms, whereas 'South' is a site in the vicinity of the Maipo River where upwelling events are rare, and with more dinoflagellate abundance than the other study sites (Fig. 6). The water was collected from a depth of 5 m, with salinity of 34.5−34.6 and temperature of 12−13 °C. The water was filtered through a 200-µm mesh and transferred to pre-acid-washed and cleaned carboys. The 15-L polycarbonate carboys were set outside and submerged in a 1 m^3 water tank with running water pumped from the sea maintaining the in situ

temperature (Fig. 7). The carboys were covered with a screen to reduce the ambient irradiance to approximately 10% of the light above the screen.

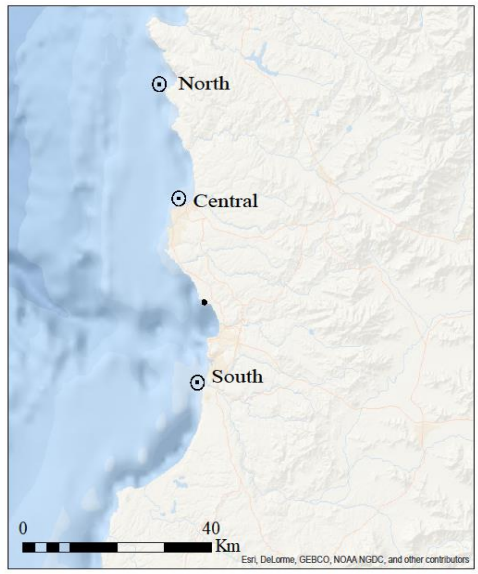


Figure 6. Sampling sites (North, Central, and South) in the Humboldt Current System experiment.

Figure 7. Experimental set up of the Humboldt Current System experiment carried out outside the Institute Las Cruces, Chile.

In this study, the effect of the phytoplankton community composition on the bacterioplankton structure was followed, using a different approach than in the GoF experiment. This approach aimed at using different N:P ratios to indirectly promote the blooming of various phytoplankton and/or bacterial communities, which could have influenced the energy transfer to other trophic levels. The various N:P ratios were achieved by the addition of nitrate at 14.4 and 3.6 µmol L^{-1} and the addition of phosphate at 0.9 and 1.6 µmol L^{-1}. Due to the existence of inorganic nutrients in the water collected the average N:P ratios in the two treatments were N:P = 10 (from herein NP 10) and N:P = 5 (from herein NP 5), respectively (Table 2). The experimental set up was a 3^2 design by the combination of the three locations ('North', 'Central' and 'South') and the two NP levels (NP 5, NP 10) and with three replicates per combination, constituting in total 18 carboys. The plankton carbon biomass (bacterioplankton and nano-microplankton) shown in this chapter correspond to samples collected on the starting day, the Chl *a* peak and final day.

Experiment	GoF 2012			GoF 2013		HCS			
Treatment	DIATOM	DINOF	CONTR1	AT, TB, BB	CONTR2	N NP 5	N NP 10	P NP 5	P NP 10
Addition	0.7	0.4	-	0.2-0.3	-	50.4	200.2	37.2	27.9

Table 2. Culture, based on Chl *a* concentrations, and inorganic nutrient (N, P) additions (µg L^{-1}) used for the Gulf of Finland experiment and Humboldt Current System experiment, respectively.

3.4 Environmental variables (I−III)

The inorganic nutrients, nitrite + nitrate nitrogen (NO_2+NO_3-N), ammonium nitrogen (NH_4-N), phosphate phosphorus (PO_4-P) and dissolved silica (DSi) were immediately analysed in the GoF experiment (I) and on the cruises (II), whereas in the HCS experiment (III) the samples for NO_3^-, PO_4^{3-} and DSi analysis were stored at -20 °C and determined immediately right after the experiment.

The Chl *a* samples were filtered onto GF/F filters and extracted in 10 mL of 96% ethanol. The samples were stored in darkness at -20 °C during the experiments (I, III), whereas during the cruises (II) the filters were kept frozen in liquid N until further determination. Immediately after the cruises, the samples were placed at room temperature for 24 h before the extraction and quantification with a spectrofluorometer.

The light and temperature were continuously recorded in the HCS experiment (III), whereas in the GoF experiment (I) they were measured each sampling day. In the case of the cruises (II), salinity, temperature, depth, Chl *a* fluorescence and dissolved oxygen were recorded *in situ,* using the conductivity, temperature, depth (CTD) sensors.

3.5 Phytoplankton related variables and analysis

3.5.1 Phytoplankton community composition (I−III)

The samples for the analysis of the phytoplankton community composition, including nano- and microzooplankton were preserved with acid Lugol's solution and stored at 4 °C in darkness until the plankton community was determined by inverted microscopy (Leitz DM IRB, Leica) and cell biovolume was converted to carbon biomass in the GoF experiment (I) and for the samples of the cruises (II). In the HCS experiment (III), the phytoplankton community was determined by flow cytometry (BD InFlux Cell Sorter equipped with 488-nm and 640-nm lasers) and by FlowCam (FluidImaging). The flow cytometry samples were fixed with glutaraldehyde (25%), and kept in liquid N until further count. The FlowCam samples were taken three times: at the start (day 0), at the Chl *a* peak based on fluorescence measurements (days 5, 7 and 9 for 'North', 'South' and 'Central', respectively) and at the end of the experiment (day 12). The samples were preserved with acid Lugol's solution and stored at 4°C until they were counted.

3.5.2 Phytoplankton bloom phase definition (I, II)

The phytoplankton and bacterioplankton bloom phase was defined for the GoF experiments (I): phytoplankton bloom phase, from the start of the experiment until nitrate depletion and with the temperature at 4 °C; bacterial bloom phase, after the nitrate depletion until the end of the experiment and temperature at 10 °C. In the case of the cruises (II), four different phases referred to Growth, Peak, Decline and Postbloom of the phytoplankton bloom phase were defined based on the Chl *a* concentration

and inorganic nutrient concentration, nitrate+nitrite for the GoF, BP, ArS, ÅS, BS subbasins and phosphate for the BoB subbasin at each station during the cruises.

The diatom/dinoflagellate (Dia/Dino) index, which indicates the ratio of diatom to dinoflagellate biomass, was calculated for the phytoplankton bloom phase in the GoF experiment (I) and for each station of the cruises (II), but using carbon biomass rather than biovolume. Values higher than 0.5 indicate diatom dominance and values lower than 0.5 indicate dinoflagellate dominance.

3.5.3 Primary production and percentage of extracellular release

The PP was estimated in the GoF experiment (I) and in the cruises (II) by measuring the incorporation of labelled sodium bicarbonate ($Na^{14}CO_3$), which was added as 0.1−0.2 µCi final activity. Three scintillation vials were incubated per treatment (I) and station (II): one in darkness and two exposed to light. After the incubation period (2 h), 100 µL of 2 M HCl were added and the vials were left without a lid for 24 h in a fume hood. The scintillation cocktail (7 mL) was then added and the ^{14}C incorporation was determined with a scintillation counter. The PP was calculated from the measured uptake of ^{14}C, knowing the total amount of isotope added and total (DIC). The DIC in each sample was measured with a high-temperature combustion infrared (IR) carbon analyser. The PP is considered gross production, due to the relatively short incubation (Sakshaug *et al.,* 1997).

The PER was calculated as the ratio of the dissolved organic fraction ($DO^{14}C$ < 0.2 µm) to the total ^{14}C fixation produced by phytoplankton ($TO^{14}C$= $PO^{14}C$+$DO^{14}C$ in the water sample without filtering), after 24 h incubations on the cruises (II). Since the PER was calculated after 24-h and the bacteria likely consumed some of the $DO^{14}C$ released, the values shown in this work are considered as net DOC production rates.

3.6 Bacterioplankton related variables and analysis

3.6.1 Bacterial secondary production (I, II)

BSP was analysed by the simultaneous incorporation of DNA-[^3H] TdR and protein-[^{14}C] Leu. Three to four replicates (1 or 10 mL) were taken and spiked with [methyl-^3H]-TdR and [^{14}C (U)]-Leu (PerkinElmer Inc., Waltham, MA, USA) at final concentrations of 14–20 nM and 100–166 nM, respectively. The isotope additions were added at saturating concentrations to ensure the incorporation of the exogenous molecules (^3H-TdR and ^{14}C Leu) and repress *de novo* synthesis pathways. One of the replicates was fixed with formaldehyde (final concentration 1.85%) and served as a blank. The samples were incubated for 2 h in darkness at 4 °C in the phytoplankton bloom phase and at 10 °C in the bacterial bloom phase in the GoF experiment (I) and at *in situ* temperature in the cruise incubations (II). The cold trichloroacetic acid (TCA) extraction method was applied, since proteins and nucleic acids are insoluble in cold TCA (Kirchman 1992). The centrifugation extraction method was used in the GoF experiment in 2013 and also in the experiments performed on the cruises, whereas in the 2012 GoF experiment the filtration method was used. The pellets/filters were stored at -20 °C until they were analysed with the scintillation counter. They were dissolved with the scintillation cocktail, and the radioactivity was determined with a Wallac WinSpectral 1414 counter. The incorporation rates (TdR and Leu) estimated as nmol L^{-1} h^{-1} were converted to carbon-based BPT and BPL as µg C L^{-1} h^{-1} with the use of several CFs:

BPT = TdR incorporation × cell CF × carbon CF,

where cell CF is the number of cells produced per mole of TdR incorporated and the carbon CF converts the cell volume in carbon units. In our studies, the cell CF used (1.4×10^{18} cells per TdR) is the value suggested by the HELCOM commission for the Baltic Sea system (HELCOM 2008) and the carbon CF used is the value suggested by Norland et al. 1993 (0.12 pg C × cell (0.06 μm^3 $cell^{-1}$)$^{0.7}$).

BPL = Leu incorporation × molecular weight Leu / (Leu per protein) × (cell carbon per protein) × ID

The BPL estimate from Leu incorporation requires fewer CFs if constant Leu content per protein (0.073) and a constant cellular carbon per protein (0.86) are assumed. Thus, the source of variation in this estimation is only in what has been called the 'isotope dilution', which corrects for Leu biosynthesis and has been suggested an average factor of 2 in coastal waters. A theoretical CF can be set at 3 kg C mol Leu^{-1} and 1.5 kg C mol Leu^{-1} with an isotope dilution of 2 or no isotope dilution, respectively (Simon & Azam 1989). In our study, the isotope dilution used was 1, according to Kirchman (2001).

3.6.2 Bacterial abundance (I, II)

BA was determined by flow cytometry from duplicate samples (1.2−1.5 mL), which were fixed with paraformaldehyde (1% final concentration) and stored at -80 °C until further analysis. The samples were stained with SYBR Green I (Sigma-Aldrich). In the 2012 GoF experiment the samples were counted with an LSRII flow cytometer and the abundance was obtained with FACS Diva software. A Partec-CUBE flow cytometer and the Flow Cytometry Standard Express 4 Flow Research Edition software were used in the GoF experiment in 2013 (I) and in the cruise samples (II).

3.6.3 Bacterial respiration (III)

The oxygen consumption was measured, using the Winkler titration method (Metrohm 848 Tritino Plus potentiometric titrator; Metrohm AG, Herisau, Switzerland). The water collected was filtered through a 0.8-µm filter and gently syphoned into 125-mL Winkler bottles that were placed in a water bath at *in situ* temperature in darkness. To determine the appropriate incubation time, a three-replicate time-series test (1, 3, 7 and 14 time points for a total of 14 days) was run on the 2014 cruise, using water samples from six different stations. Once the proper incubation time was set, 7 days, duplicate samples were incubated on the 2015 and 2016 cruises at each station. The oxygen concentration was determined on the sampling date (day 0) and at the end of the incubation (7 days). The oxygen concentration decrease was converted to CO_2 production, using a respiratory quotient of 1.

3.6.4 Bacterial community composition (I−III)

The samples for BCC were collected at the start of the experiment (day 0), at the Chl *a* peak, at the BSP peak for the GoF experiment in 2013 and at the end of the experiment for the 2012 GoF and HCS experiments (I, III). In the 2012 GoF experiment, the three replicates were pooled by treatment, and thus there were seven samples in total: one sample from the natural community at day 0, one per treatment (three samples) at the Chl *a* peak and one per treatment (three samples) at the end of the experiment. In the 2013 GoF experiment, one replicate per carboy (three samples per treatment) was analysed on the three sampling days mentioned above (12 x 3 = 36 samples). In the HCS experiment (III), the BCC was analysed in one replicate from each of the three communities ('North', 'Central' and 'South') at the start of the experiment, from each of the three replicates per treatment (NP 5 and NP 10) at the Chl *a* peaks (day 5 in 'North', day 7 in 'South' and day 9 in 'Central') and end of the experiment (3 + (18 x 2) = 39). During the cruises (II), the BCC was analysed from one sample per station taken at a depth of 3 m.

The water (500 mL) was gravity-filtered onto sterile 0.2-µm pore-size cellulose ester filters and stored at -80 °C for further analysis. Later, the DNA was extracted, using the Power Soil DNA isolation kit (Qiagen N.V., Venlo, The Netherlands). The V1 to V3 hypervariable region of the 16S rRNA gene was amplified in two-step polymerase chain reactions (PCR), using the universal bacterial primers F8 (Chung *et al.,* 2004) and R492 (Edwards et al., 1989). Multiplex sequencing was performed with the paired-end Illumina MiSeq platform for all the samples (I−III).

3.6.5 Postprocessing of sequencing data (I−III)

The postprocessing of the sequencing samples was done with the Illumina MiSeq (Table X). Primers were removed using Cutadapt. The paired-end reads were merged, using PEAR software. In general, quality filtering (> 400 bp, maximum expected error 1), chimera checking and operational taxonomic unit (OTU) clustering were done with the UPARSE pipeline. Taxonomic classification of the OTUs was done with Silva at 97% of similarity in Mothur for the GoF and HCS experiments (I, III) and at 99% of similarity for the cruises (II). Chloroplasts, mitochondria and singletons were removed with Silva v123, based on the phylogenetic classification. The libraries were normalized with metagenome Seq, using R for the GoF experiments (I), whereas rarefaction was applied in the sequencing libraries on the cruises (II). No normalization method was applied in the sequencing libraries in the HCS experiment. From the rarefied data (cruise data), samples with less than 7000 reads (four samples) were discarded (II). Further analyses were done from the final number of samples and final number of OTUs (Table 3). The final figures of the BCC are formed by the groups that contribute to more than 0.8% in the experiments (I, III) and more than 0.5% in the cruises (II).

	I	II	III
Raw reads 16S rRNA gene	~ 13 million	-	~ 6.2 million
Merged reads after quality filtering	1.8 million	4.22 million	1.9 million
OTUs after removal Mitochondria/chloroplast	1720	2247	916
Normalization	+	-	-
Rarefaction	-	+	-
Final number of samples	7 (2012), 25 (2013)	122	39
Final number of OTUs	1720	2128	916

Table 3. Summary of the main results obtained from the pipelines used in the three articles included in this PhD thesis.

3.7 Statistical and multivariable analysis (I−III)

The treatment effects were statistically analysed with analysis of variance (ANOVA), using Tukey's b as the post-hoc test (I). The effect of the various phytoplankton additions as treatments was tested in the parameters BPL, BPT and BA, in the GoF experiment (I). To include the temporal effect in the analysis, the variables examined were transformed to a cumulative value, adding each value to the previously determined value. Linear regression was done for all replicates, and the slope of the regression was used for the ANOVA analysis. Differences in the diatom and dinoflagellate carbon biomass between the phytoplankton bloom phases (II) were tested the non-parametric Wilcoxon rank-sum test. In all the cases, the homogeneity of the variance was tested with Levene's test, and in case of unequal variance the data were transformed or ranked.

The treatment effects on the BCC in the 2013 GoF experiment and HCS (I, III) were tested with repeated-measures permutational ANOVA (PERMANOVA), using pairwise comparison (Anderson 2001, McArdle & Anderson 2001). A total of 9999 permutations were performed. The homogeneity of dispersion was tested with permutational multivariate analysis of dispersion (Anderson 2006), using the distance to the centroids.

Multivariable analyses were performed in the bacterial community to determine their distribution patterns (I−III) and the environmental variables shaping the bacterial structure and dynamics (II). For this, non-metric multidimensional scaling (NMDS) plots were constructed from the previous Bray-Curtis dissimilarity matrix calculated (I−III). The NMDS plots visualized the level of dissimilarity between the bacterial communities in each treatment (I, III) and also the alpha (richness) and beta diversity of the OTUs (II). The significant environmental variables affecting the bacterial community structure were determined by a redundancy analysis (RDA), with forward selection in R (II). Previously, collinearity was tested to exclude the highly correlated variables by using the variance inflation factors (VIF). The

variables with VIF < 10 remained for further analyses. The RDA forward selection analysis included an ANOVA test in each step (permutation test for RDA under reduced model, permutations: free, number of permutations: 9999) to identify the significant environmental variables ($p < 0.05$). Spearman correlations were applied to obtain the relationship (+ or -) between the main bacterial groups, with the significant environmental variables obtained with the RDA (II). The Spearman correlation was applied to compare the main bacterial groups with the bacterial activity. Further correlations between the bacterial and phytoplankton community composition were tested with the Mantel test (II).

4 RESULTS

4.1 Phytoplankton bloom dynamics (I−III)

The initial phytoplankton communities found at the sampling sites in the GoF and HCS experiments, as well as during the cruises in the various subbasins in the Baltic Sea, differed in carbon biomass, community composition and bloom phase, due to the environmental conditions existing at the sampling time.

4.1.1 Phytoplankton bloom development in the Gulf of Finland (GoF) experiment (I)

The water for the GoF experiments was collected in winter (March 2012 and February 2013), when the surface water was still covered by ice (> 30 cm) and light availability in the water column was low. The water sampled was characterized by high inorganic nutrient concentrations (NO_2+NO_3-N > 80 µg L^{-1}; PO_4-P > 27 µg L^{-1}), low Chl *a* concentration (< 0.1 µg L^{-1}), and low PP < 0.25 µg C L^{-1} h^{-1}, which indicates that the phytoplankton were in the pre-bloom phase at the time of the sampling (Table 4).

The differences in the initial inoculum concentration of the monocultures (Table 2) clearly affected the phytoplankton bloom in terms of carbon biomass, community composition and quickness of bloom development. The inoculum was 3.5-fold higher in 2012 than in 2013. Once the experiment was set at 4 °C, and under the experimental conditions designed for the study, the addition of the diatom and dinoflagellate monocultures to the respective treatments resulted in the development of the phytoplankton bloom phase in 10−19 days. Later, the inorganic nutrient concentrations (nitrate and phosphate) decreased (< 2 µg L^{-1}) and the Chl *a* and PP peaks were observed. The temperature was raised to 10 °C and the bacterial bloom phase was promoted. The DSi was higher (> 170 µg L^{-1}) in the dinoflagellate dominated treatments than in the diatom dominated treatments. The Chl *a* and PP peaks in 2012 in both, the DIATOM and DINOF treatments, occurred on day 10, whereas in the AT, TB and BB treatments in 2013, they were between days 13 and 15 (Table 4). In the controls, the peaks occurred on day 19 (CONTR1) and day 15 (CONTR2). The Chl *a* concentrations were larger in the 2013 experiment (> 70 µg L^{-1}) than in 2012, which presented the lowest Chl *a* value in the DINOF treatment. This treatment also recorded the lowest PP (22.55 µg C L^{-1} h^{-1}), whereas in the BB treatment the PP was high, similar to the AT and TB treatments. By the end of the experiments, the inorganic nutrients were depleted and the Chl *a* concentration and PP decreased. The lowest DSi concentration was observed in the BB treatment at the end of the experiment, whereas the highest value (> 300 µg L^{-1}) was in the DINOF treatment.

The natural plankton communities without inoculation of any monoculture (CONTR1-2) were dominated by diatoms (diatom/dinoflagellate (Dia/Dino) index > 0.90, Table 4), such as *Thalassiosira levanderi and Achnanthes taeniata* at the Chl *a* peaks (I). These species, together with *Chaetoceros wighamii*, *Skeletonema marinoi* and *T. baltica*, were present in the treatments with diatom additions (DIATOM, AT, TB) on this day, and largely in the experiment of 2012 with carbon biomass > 1.8 mg C L^{-1} (Fig. 8). The dinoflagellate complex predominated in the DINOF treatment (2012) at the Chl *a* peak, whereas in the BB treatment (2013) the diatom carbon biomass was higher than the carbon biomass of the dinoflagellates.

	Treatment	Temp (ºC)	NO2-NO3-N µg L^{-1}	PO4-P µg L^{-1}	DSi µg L^{-1}	Chl *a* µg L^{-1}	PP* µg C L^{-1} h^{-1}	BPT/PP	BPL/PP	Dia:Dino index
GF experiment										
Day 0										
2012		<1	97.47 ± 1.68	31.21 ± 0.09	623.83 ± 0.33	0.07 ± 0.03	0.25 ± 0.18	1.11 ± 0.54	0.60 ± 0.33	-
2013		<1	80.20 ± 3.29	27.40 ± 0.24	520.23 ± 0.79	0.02 ± 0.00	0.24 ± 0.07	0.65 ± 0.28	0.26 ± 0.11	-
Day Chl a peak										
2012										
Day 19	CONTR1	4	0.97 ± 0.20	0.99 ± 0.13	17.70 ± 0.32	23.03 ± 1.59	33.60 ± 12.16	0.03 ± 0.01	0.02 < 0.00	0.96
Day 10	DIATOM	4	1.10 ± 0	2.89 ± 0.34	162.87 ± 37.87	20.60 ± 6.12	49.49 ± 3.16	0.03 < 0.01	0.01 < 0.00	0.99
Day 10	DINOF	4	2.12 ± 2.92	1.74 ± 0.35	553.90 ± 1098	9.87 ± 0.48	22.55 ± 1.96	0.02 ± 0.01	0.01 < 0.00	0.15
2013										
Day 15	CONTR2	4	1.70 ± 0.10	2.23 ± 0.48	62.67 ± 23.89	42.83 ± 0.74	70.86 ± 0.34	<0.00	<0.00	0.94
Day 15	AT	4	2.17 ± 0.20	1.53 ± 0.21	102.20 ± 5.80	39.77 ± 1.29	50.99 ± 2.18	0.01 < 0.00	0.02 < 0.00	1.00
Day 15	TB	4	1.97 ± 0.03	1.80 ± 0.17	103.27 ± 21.04	45.80 ± 1.99	48.82 ± 3.39	0.01	0.02	0.98
Day 15	BB	4	2.03 ± 0.03	2.03 ± 0.52	171.93 ± 10.73	54.03 ± 0.35	49.00 ± 1.86	0.00	0.01	0.69
Day end										
2012	CONTR1	10	0.87 ± 0.12	0.47 ± 0.20	34.24 ± 15.09	1.97 ± 0.52	0.87 ± 0.31	2.36 ± 0.91	1.64 ± 0.67	-
	DIATOM	10	0.53 ± 0.03	0.17 ± 0.17	96.50 ± 26.20	2.20 ± 0.67	0.36 ± 0.13	9.01 ± 3.20	4.71 ± 0.64	-
	DINOF	10	0.60 ± 0.06	0.47 ± 0.20	303.36 ± 42.13	0.77 ± 0.20	0.55 ± 0.19	7.19 ± 0.49	1.67 ± 0.99	-
2013										
	CONTR2	10	0.87 ± 0.13	0.25 ± 0.25	21.92 ± 1.46	7.77 ± 0.98	0.62 ± 0.21	1.31 ± 0.20	0.75 ± 0.01	-
	AT	10	0.85 ± 0.13	0.43 ± 0.28	36.04 ± 4.25	6.67 ± 0.96	1.72 ± 0.30	1.63 ± 0.08	2.10 ± 0.27	-
	TB	10	0.27 ± 0.14	0.25 ± 0.15	22.69 ± 2.30	5.97 ± 1.01	0.78 ± 0.18	1.16 ± 0.11	1.23 ± 0.30	-
	BB	10	0.50 ± 0.14	0.49 ± 0.41	17.48 ± 0.38	10.38 ± 0.49	2.56 ± 0.38	0.94 ± 0.08	1.27 ± 0.09	-

Table 4. Environmental variables measured in the Gulf of Finland experiment in 2012 and 2013 on day 0, day of the Chl *a* peak and day end. The values indicate average and standard error. The Chl *a* peaks in 2012 were on day 10 in the DIATOM and DINOF treatments and on day 19 in CONTR1; in 2013 they were on day 15. The day of the PP peak was on day 13, indicated with the (*) symbol. The Dia/Dino index was calculated for the entire phytoplankton bloom phase, 19 days in 2013 and 15 days in 2013.

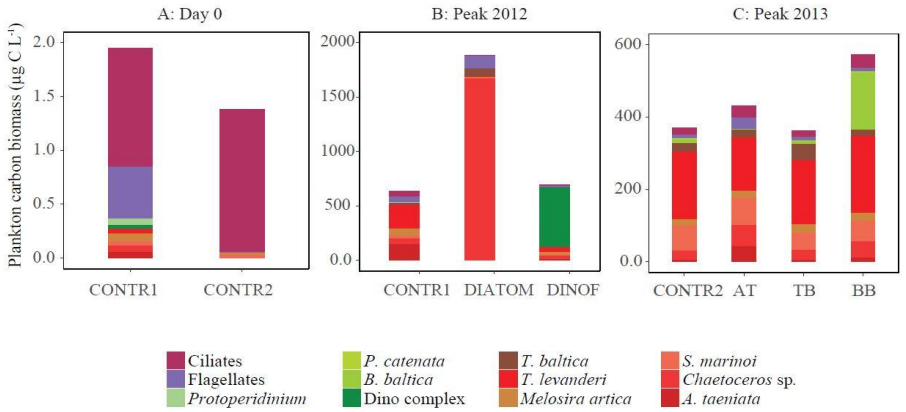


Figure 8. Microplankton (phyto- and zooplankton) carbon biomass A) at the start of the experiment (CONTR1 and 2) and at the Chl *a* peaks; B) Peak 2012 and C) Peak 2013) in the Gulf of Finland experiments.

4.1.2 Phytoplankton bloom dynamics in the Baltic Sea (II)

The water was collected on the different cruises during the spring season. However, the environmental conditions between and within the sampling years in the various subbasins were highly variable. A salinity gradient, ranging from 2.2 to 7.7, was observed from the northernmost BoB to the southernmost BP station (Table 5). The temperature and the inorganic nutrient concentration also varied between the cruises, shaping the phytoplankton community found in our study (Fig. 9).

In 2013, the sampling was conducted immediately after the ice had melted, and the temperature was low (< 2.7 °C), with inorganic nutrients still available (nitrate > 80 µg L^{-1}; phosphate > 27 µg L^{-1}) in the early bloom (Table 5). In the rest of the years, the temperature was higher (> 4 °C) and the nitrate was depleted, except in the first half of the cruise in 2016 and in the BoB, where the temperature and nitrate concentrations were similar to those in the cold 2013 year. In the BoB, the phosphate was depleted (~ 0.1 µg L^{-1}) instead. Regarding the concentrations of DSi, the lowest measured was in the GoF in 2014 (63.85 µg DSi L^{-1}) and the highest (1554.61 µg L^{-1}) was measured in the Kemi River plume in the northernmost BoB.

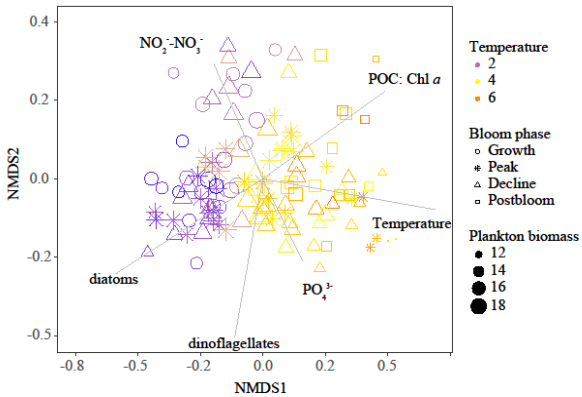


Figure 9. Nonmetric multidimensional scaling (NMDS) plot based on Bray-Curtis distances of plankton taxonomic composition during the sampling years. The colour indicates the salinity gradient, shape the phytoplankton bloom phase and size the log-transformed plankton carbon biomass at each station. The vectors indicate the significant environmental variables shaping the plankton community composition. N = 122, stress = 0.195.

	Subbasins	Bloom Phase	Temperature (ºC)	Salinity	NO2-NO3-N µg L^{-1}	PO4-P µg L^{-1}	DSi µg L^{-1}	Dia:Dino index	POC:Chl *a*	BPT/PP	BPL/PP	BR µg C L^{-1} h^{-1}
Cruise												
2013	GoF (39)	G, P, D	**0.76**-1.98	5.09-6.38	**0**-82.03	6.19-12.39	220.23-554.08	0.30-0.86	33.36-52.47	0.01-0.03	0.01-0.04	-
April	BP (11)	G, P	1.62-2.72	5.97-7.24	0-29.14	1.86-10.84	232.30-400.01	0.09-**0.94**	38.96-100.35	0.02-0.08	0.01-0.07	-
	ÅS (4)	G, P	1.29-1.59	5.45-6.16	0-36.28	0.62-10.78	250.56-367.95	0.36-0.74	44.54-56.39	0.03	0.03	-
2014	GoF (9)	D, PB	4.25-5.18	5.03-6.01	0-3.64	3.72-10.22	**63.85**-255.62	0.11-0.29	49.43-80.00	0.02-0.23	0.01-0.08	0.11-0.26
May	BP (2)	D, PB	4.62-5.69	6.56-6.63	0-5.04	8.67-10.43	255.63-334.27	**0.01**-0.36	106.82-176.30	0.11-0.26	0.03-0.07	0.06-**2.78**
	ArS (4)	D, PB	4.58-5.17	5.90-6.29	0-5.60	6.19-10.22	168.44-227.53	0.05.0.49	88.28-208.84	0.24-0.43	0.05-0.09	0.12
2015	GoF (5)	D, PB	3.91-5.35	4.96-5.86	0-0.28	5.57-11.15	154.50-555.08	0.12-0.40	75.28-119.28	0.02-0.07	0.01-0.03	0.69-0.99
May	ArS (6)	P, PB	4.41-6.09	5.75-6.38	0-3.22	1.86-10.22	157.30-311.80	0.04-0.32	58.81-190.20	0.01-0.06	0.01-0.02	0.39-2.08
	ÅS (4)	D	5.03-**6.22**	5.41-6.06	0-0.14	1.86-6.81	188.20-244.38	0.17-0.68	76.75-108.31	0.02-0.05	0.01-0.05	0.48-0.49
	BP (4)	D, PB	4.52-5.69	5.72-6.56	0-6.16	8.36-12.39	213.48-331.46	0.09-0.33	83.49-149.96	0.02-0.07	0.01-0.03	2.08
	BS (10)	P	3.59-4.16	5.12-5.54	0-5.32	**0**-6.50	227.53-356.74	0.48-0.90	31.93-82.12	0.01-0.04	0.00	**0.05**-0.57
	BoB (9)	P, D	1.35-4.73	**2.20**-5.09	11.21-**104.65**	0-0.62	544.95-**1564.61**	0.36-0.68	70.71-**223.37**	0.05-0.69	0.01-0.44	0.35-0.68
2016	GoF (4)	G, P, D	1.56-2.89	4.95-5.20	0-93.00	10.65-**18.89**	343.04-536.32	0.13-0.77	**25.85**-57.10	0.01-0.03	0.02	0.18-0.58
April	BP (20)	G, P, D, PB	2.47-5.90	5.92-**7.77**	0-26.41	4.89-15.86	330.70-485.51	0.01-0.58	44.49-260.31	0.01-0.11	0.01-0.06	0.05-1.72
	ÅS (4)	P	2.84-3.28	5.51-5.68	0-2.44	3.62-5.64	437.42-510.48	0.61-0.80	58.41-67.04	0.01	0.01	0.13-0.39

Table 5. Environmental variables measured during the four cruises (2013−2016) in the Baltic Sea. The subbasins covered during the respective cruises were Gulf of Finland (GoF), Baltic Proper (BP), Åland Sea (ÅS), Archipelago Sea (ArS), Bothnian Sea (BS), Kvarken and Bay of Bothnia (BoB). The bloom phases defined in the cruises are Growth (G), Peak (P), Decline (D) and Post bloom (PB). The values represent the range between the minimum and maximum values, in bold, within the four years. The Dia/Dino index was calculated for each station.

The phytoplankton bloom in the cold year (2013), from Growth to Decline, showed high carbon biomass of diatoms and dinoflagellates (> 200 µg C L^{-1}, Fig. 10) and higher carbon biomass of *Ebria tripartita* than in the other cruises (Fig. 10A), except in the southernmost BP stations with values of carbon biomass < 50 µg C L^{-1}. The diatom- and dinoflagellate-dominant species were the same as in the GoF experiment (Fig. 10A). However, the dinoflagellate complex was not identified at species level during the cruises due to the lack of a standardized identification methodology. The phytoplankton bloom in the remaining years was more advanced than in 2013, with stations in the Postbloom phase and largely dominated by dinoflagellates, the mixotrophic ciliate *Mesodinium rubrum*, heterotrophic ciliates and heterotrophic nanoflagellates (HNFs) in 2014 and 2016. The 2014 cruise showed the lowest plankton carbon biomass (< 50 µg C L^{-1}) observed, comparable to some stations in the BoB and the southernmost BP stations in 2013.

The highest values of Chl *a* concentration and PP (Chl *a* > 20 µg L^{-1} and PP > 60 µg C L^{-1} h^{-1}) were observed in the cold 2013 cruise and in general during the Growth and Peak phases of the bloom in the GoF, BP, ÅS and the BS in all the cruises, coinciding with the stations that showed high carbon biomass (Fig. 10C). The PER was higher (> 8%) in the Decline and Post bloom phase in the BP and the BoB, and opposite to the PP. In the BoB, the Chl *a* and PP values were low (< 7 and < 5, respectively). The BS showed high Chl *a* concentration and PP values (> 10 and > 19, respectively) with a phytoplankton bloom in the Peak phase and largely dominated by the diatom *T. baltica* and the mixotrophic ciliate *M. rubrum*.

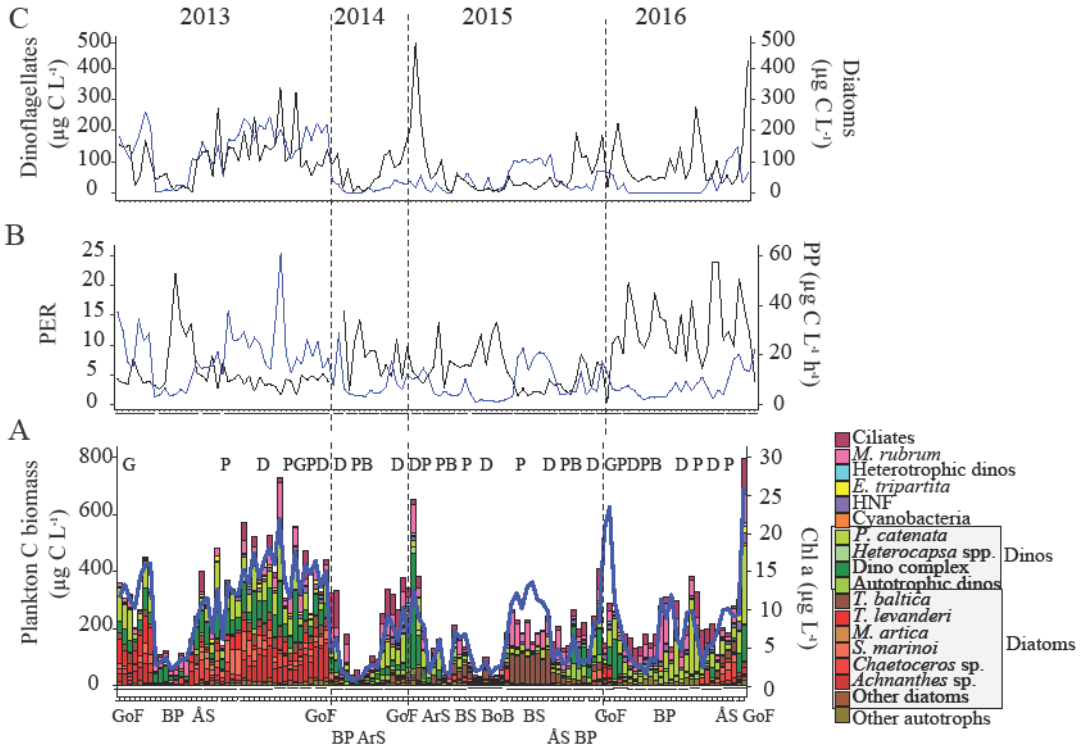


Figure 10. Phytoplankton dynamics during the sampling cruises, from station 1 in 2013 to station 126 in 2016. A) Plankton carbon biomass and Chl *a* concentrations in the four cruises. In the legend, diatoms and dinoflagellates (dinos) species are highlighted by boxes. The x-axes indicate the bloom phases: Growth (G), Peak (P), Decline (D) and Post bloom (PB) phases; and sub-basins: Gulf of Finland (GoF), Baltic Proper (BP), Åland Sea (ÅS), Archipelago Sea (ArS), Bothnian Sea (BS), and Bay of Bothnia (BoB). B) Total carbon biomass of dinoflagellates (black) and diatoms (blue). C) Primary production (PP, blue) and percentage of extracellular release (PER, black) in the various subbasins.

4.1.3 Phytoplankton bloom development in the Humboldt Current System experiment (III)

The water was collected from three different upwelling locations in the HCS off the Chilean coast during the austral summer (March). The locations: 'North', 'Central' and 'South' differed in their phytoplankton community composition. At the time of water collection, 'North' showed the highest plankton carbon biomass (> 600 µg C L^{-1}), dominated by the diatom *Thalassiosira* spp., whereas the other two locations had lower carbon biomass (< 106 µg C L^{-1}) and were dominated by diatoms (*Thalassiosira* spp.) in 'Central' and dinoflagellates (*Prorocentrum* sp.) in 'South' (Fig. 11). The locations also differed in their initial Chl *a* concentration and inorganic nutrient concentrations (Table 6). 'North' showed the highest Chl *a* (> 27 µg L^{-1}) and the lowest nitrate and phosphate concentration, but high enough (nitrate > 60 µg L^{-1}, phosphate > 31 µg L^{-1}) for the phytoplankton growth, whereas 'Central' contrasted, with the lowest Chl *a* (3.48 µg L^{-1}) and highest nutrient concentration. 'South' showed intermediate values. Thus, the initial phytoplankton communities at the different locations were at different growth phases. The initial DSi concentration was similarly high in the three locations (> 340 µg L^{-1}).

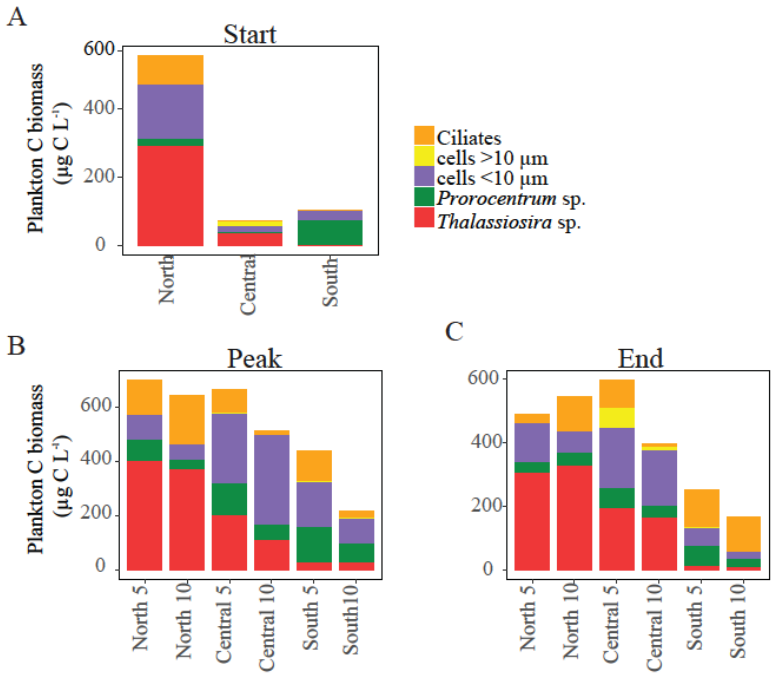


Figure 11. Plankton carbon biomass of the Humboldt Current System experiment in the three locations 'North', 'Central', and 'South' A) at the start, and also B) at the chlorophyll *a* peak and C) end day in their respective nitrogen:phosphorus treatments (NP 5 and 10).

After the addition of the nitrate and phosphate to the treatments, the phytoplankton growth was enhanced and the nitrate was consumed (< 2 µg L^{-1}). The phosphate remained available throughout the experiment and more available in the NP5 treatment; *e.g.* in 'South NP5' the phosphate showed larger values (> 60 µg L^{-1}) than under initial conditions by the end of the experiment. The phosphate decreased more throughout the experiment in 'North NP 10' and 'Central NP 10' than did the corresponding NP5 treatments. The DSi decreased, first in 'North' at the Chl *a* peak and at the end of the experiment in 'Central,' whereas in 'South' it also decreased but remained higher than in the other study sites. At the Chl *a* peak, an increase in the plankton carbon biomass in the three locations and in the Chl *a* concentrations in 'North' and 'Central' was observed (Table 6).

	Subbasins	Treatment	NO_2-NO_3-N µg L^{-1}	PO_4-P µg L^{-1}	DSi µg L^{-1}	Chl *a* µg L^{-1}	Pico-phytoplankton Cell mL^{-1}	Nano-phytoplankton Cell mL^{-1}	Meso-zooplanton Indiv L^{-1}
HCS experiment									
Day 0									
	NORTH	-	66.77	31.90	340.65	27.39	-	-	-
	CENTRAL	-	169.44	47.34	402.76	3.48	-	-	-
	SOUTH	-	84.87	34.64	360.06	15.32	-	-	-
Day Chl a peak									
Day 5	NORTH	5	0.49 ± 0.49	37.64 ± 1.81	59.82 ± 35.64	19.62 ± 0.56	$4x10^4 \pm 4x10^3$	$2x10^4 \pm 9x10^2$	-
		10	1.19 ± 0.21	26.86 ± 1.95	88.73 ± 20.21	42.08 ± 7.87	$3x10^4 \pm 5x10^3$	$1x10^4 \pm 1x10^3$	-
Day 9	CENTRAL	5	0.97 ± 0.44	35.50 ± 4.50	101.75 ± 23.85	20.63 ± 2.71	$7x10^4 \pm 2x10^4$	$5x10^4 \pm 2x10^4$	-
		10	2.34 ± 1.68	24.44 ± 1.28	56.18 ± 0.01	33.44 ± 11.49	$6x10^4 \pm 9x10^3$	$7x10^4 \pm 2x10^3$	-
Day 7	SOUTH	5	0.95 ± 0.48	59.25 ± 1.91	232.36 ± 23.15	6.83 ± 2.27	$5x10^4 \pm 8x10^3$	$5x10^4 \pm 3x10^3$	-
		10	1.40 ± 0.81	24.97 ± 1.01	84.29 ± 0.02	10.29 ± 4.06	$1x10^5 \pm 5x10^3$	$3x10^4 \pm 3x10^3$	-
Day end									
	NORTH	5	0.44 ± 0.44	25.91 ± 1.71	113.60 ± 15.65	6.32 ± 0.39	$1x10^4 \pm 3x10^3$	$9x10^3 \pm 3x10^3$	36 ± 14
		10	1.6 ± 0.34	19.49 ±0.70	103.67 ± 25.26	11.11 ± 1.31	$1x10^4 \pm 7x10^2$	$9x10^3 \pm 8x10^2$	59 ± 21
	CENTRAL	5	1.00 ± 0.40	27.93 ± 3.17	56.18 ± 0.01	13.30 ± 1.39	$3x10^4 \pm 9x10^4$	$3x10^4 \pm 1x10^{4*}$	20 ± 3
		10	1.40 ± 0.81	23.61 ± 0.93	57.12 ± 33.25	12.20 ± 1.78	$7x10^4 \pm 3x10^4$	$5x10^4 \pm 5x10^{3*}$	31 ± 5
	SOUTH	5	1.69 ± 0.29	62.02 ± 2.66	240.91 ± 8.39	4.38 ± 0.26	$2x10^5 \pm 2x10^{4*}$	$1x10^4 \pm 1x10^3$	100 ± 12
		10	1.79 ± 0.51	27.30 ± 0.52	126.07 ± 8.12	3.64 ± 0.61	$9x10^4 \pm 6x10^{3*}$	$3x10^4 \pm 7x10^3$	79 ± 29

Table 6. Environmental variables measured in the Humboldt Current System experiment on day 0, day of chlorophyll *a* peak and day end. The values indicate average and standard error. The Chl *a* peaks were on day 5 in 'North', day 7 in 'South' and day 9 in 'Central'.

In ‘South’, the Chl *a* concentration decreased throughout the experiment and the increase in carbon biomass was lower than in the other communities. By the end of the experiment, the Chl *a* concentration and carbon biomass decreased more in ‘North NP5’. This carbon biomass was dominated by diatoms throughout the experiment, whereas in ‘Central’, flagellates < 10 µm (nanophytoplankton) significantly increased compared with the other locations (Fig. 11, table 6, Tukey; day 12, $p < 0.006$). In the communities with more diatoms (‘North’ and ‘Central’) there were also more heterotrophic dinoflagellates (*Protoperidinium* sp.) than in ‘South’ (Tukey; $p < 0.001$). In this location, the increase in carbon biomass was observed mainly in the treatment ‘NP5’ and was due significantly to picoeukaryotes (Tukey; $p = 0.006$). Ciliates also increased in ‘South’ together with a significantly increase in mesozooplankton (Tukey; $p < 0.005$) compared with the other locations, whereas the initial dominant dinoflagellates decreased throughout the experiment.

4.2 Bacterioplankton structure and metabolic activity during phytoplankton blooms

4.2.1 Bacterial production, abundance and community composition in the Gulf of Finland experiment (I)

The BSP, measured as thymidine (BPT) and leucine (BPL) incorporation, increased throughout the experiments and largely in the experiment of 2012 (Fig. 12). During the phytoplankton bloom phase, the BSP increased slightly (< 1 µg C L^{-1} h^{-1}) in both experiments, but increased significantly in the AT treatment compared with CONTR2 in 2013 (Tukey’s b, BPT and BPL $p < 0.05$). After the nutrient depletion and increase in temperature to 10 ºC, the BPT was > 3 µg C L^{-1} h^{-1} and the BPL was > 5 µg C L^{-1} h^{-1} in the DIATOM treatment during the bacterial bloom phase. In addition, the increase in BPL was significantly higher in the DIATOM, AT and TB treatments, whereas the BPL and BPT were significantly lower in the DINOF and BB treatments (Tukey’s b, $p < 0.05$) than in the respective controls (CONTR1-2).

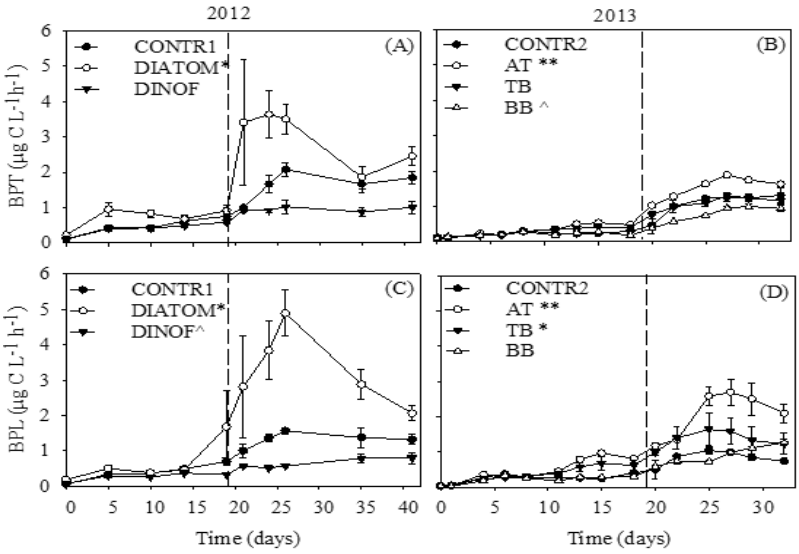


Figure 12. Bacterial production based on thymidine (BPT) and leucine (BPL) incorporation in the Gulf of Finland experiment throughout the experiments in 2012 (A, C) and 2013 (B, D). The dashed line separates the phytoplankton and bacterioplankton bloom phases. Significant differences between the treatments and the controls are indicated with * and ^ symbols in the bacterial bloom phase. Two asterisks also indicate significant differences in the phytoplankton bloom phase.

The BPT-L:PP ratio was > 0.26, indicating bacterial activity already in the Prebloom phase. At the Chl *a* peak, the ratios were < 0.03, whereas at the end of the experiment attained values > 9 (BPT:PP) and > 4 (BPL:PP) in the DIATOM treatment (Table 4).

The BA response differed between both experiments during the phytoplankton bloom phase: in 2012, the BA increased and in 2013, the BA was stable and decreased by the end of this phase (Fig. 13). In the bacterial bloom phase, the BA peaked in the DIATOM treatment (~ 7 x 10^6 cells ml^{-1}) on day 24 and decreased thereafter, whereas in the CONTR1 and DINOF treatments it continued increasing towards the end of the experiment, but to a lesser extent. In the 2013 bacterial bloom phase, the BA started to increase on day 22, with higher abundance in the AT treatment, which constituted half of the abundance in the 2012 experiment. The BA was significantly lower in the BB treatment than in the CONTR2 (Tukey's b, $p < 0.05$).

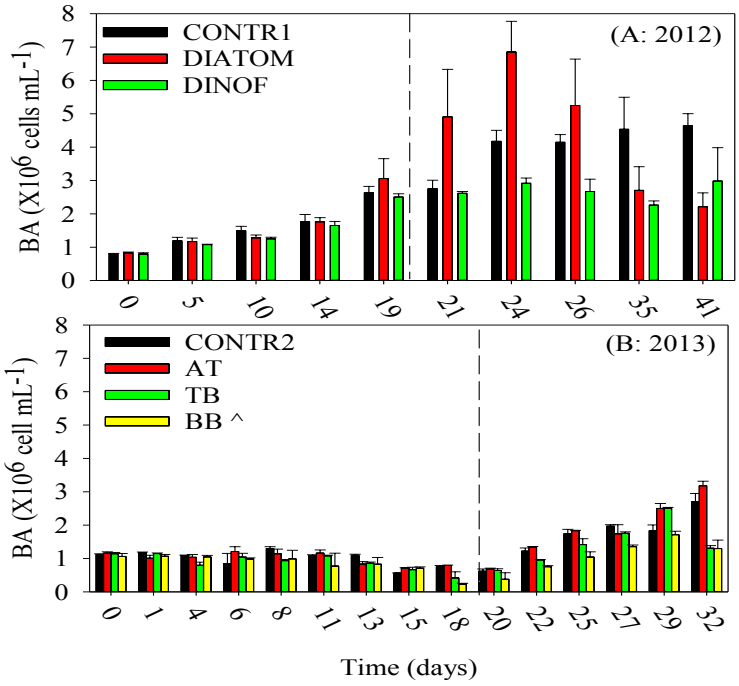


Figure 13. Bacterial abundance (BA) throughout the experiments in 2012 (A) and 2013 (B). The dashed line separates the phytoplankton and bacterioplankton bloom phases. The symbol ^ indicates significant differences compared with the control.

The BCC differed between the treatments, becoming more dissimilar throughout the experiments (Fig. 14). In the 2012 experiment, the BCC of the DIATOM treatment was largely dissimilar, compared with the DINOF treatment by the end of the experiment, but no statistical test was performed, due to the lack of replicates. In the 2013 experiment, the BCC was clustered by treatments and significant differences were found between the AT and TB treatments compared with the CONTR2 (PERMANOVA, CONTR2 vs. AT: $p = 0.027$; CONTR2 vs. TB: $p = 0.02$), whereas no significant differences were found between the CONTR2 and the BB treatment (PERMANOVA, CONTR2 vs. BB: $p > 0.05$).

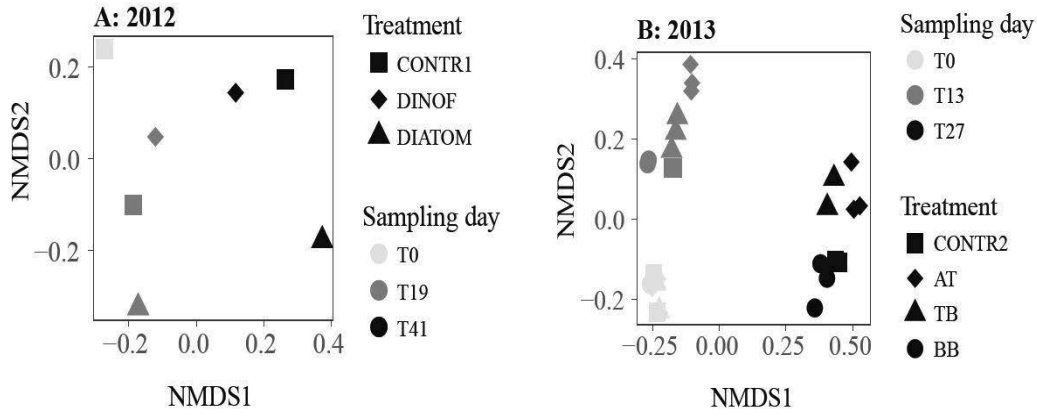


Figure 14. Nonmetric multidimentional scaling plots (NMDS) based on Bray-Curtis distances of bacterial community structure in the Gulf of Finland experiments; A) 2012 and B) 2013. The colour-code indicates the sampling day: start (T0), Chlorophyll *a* peak (day 19 in 2012 and day 13 in 2013), and bacterial production peak (day 27 in 2013) and end of the experiment (day 41 in 2012). The dot shape indicates the controls and treatments in each experiment. In 2102, N = 1 and stress value = 0.019; in 2013, N = 3 and stress value = 0.049.

There were some similarities in both experiments: the water collected was dominated by Alphaproteobacteria (SAR 11 and *Rhodobacteraceae*), with a relative abundance of ~ 50 % of the OTUs, and decreased throughout the experiment, whereas the relative abundance of Flavobacteriia peaked either at the Chl *a* peak (2012) or at the BSP peak (2013, Fig. 15). The classes Flavobacteriia, Actinobacteria (hgcI clade) and Acidimicrobiia (CL500-29 marine group) in both experiments and the classes Beta-Gammaproteobacteria in 2013 were also present in the water collected. At the 2012 Chl *a* peak, Flavobacteriia predominated in the community, which was largely dominated in the DIATOM treatment by the genus *Flavobacterium* (~ 43 %), whereas in the CONTR1 and DINOF treatments, part of the community was dominated by the NS3a marine group (Fig. 15A). The relative abundance of Gammaproteobacteria also increased further in the DIATOM treatment and in the CONTR1. By the end of the experiment, Betaproteobacteria (genus *Hydrogenophaga* ~ 20 %) also increased in the DIATOM treatment, whereas Cyanobacteria (genus *Synechococcus*) increased in the CONTR1 and DINOF treatment, and SAR 11 reverted its dominance. Actinobacteria increased in all the treatments and was represented by the hgcI clade in the CONTR1 and DINOF treatments and by the genus *Cadidatus Aquiluna* in the DIATOM treatment. Within Flavobacteriia, *Polaribacter* became the predominant genus by the end of the 2012 experiment. In the experiment of 2013, the bacterial community was dominated by transient Betaproteobacteria (~ 30 % of the OTUs) at the Chl *a* peak and represented by various genera: the BAL58 marine group in all treatments, *Albidiferax* in the CONTR2, AT and TB treatments, which also presented *Hydrogenophaga* (Fig. 15B). In addition, Gammaproteobacteria (genus *Pseudomonas*) appeared in the AT and TB treatments and accounted for ~ 20 % of the OTUs, whereas Epsilonproteobacteria (genus *Arcobacter*) appeared in all the treatments and was vastly more abundant in the CONTR2 (~12 %) and BB treatment (~ 27 %) compared to the AT and TB treatments. At the BSP peak, the bacterial community shifted to the predominance of Flavobacteriia; the genus *Flavobacterium* accounted for 25−40 % of the OTUs in all the treatments and the NS3a marine group accounted for 18−25 % in the CONTR2 and BB treatment. In addition, Cytophagia (genus *Algoriphagus*) increased in the AT and TB treatments (12−20 % of the OTUs).

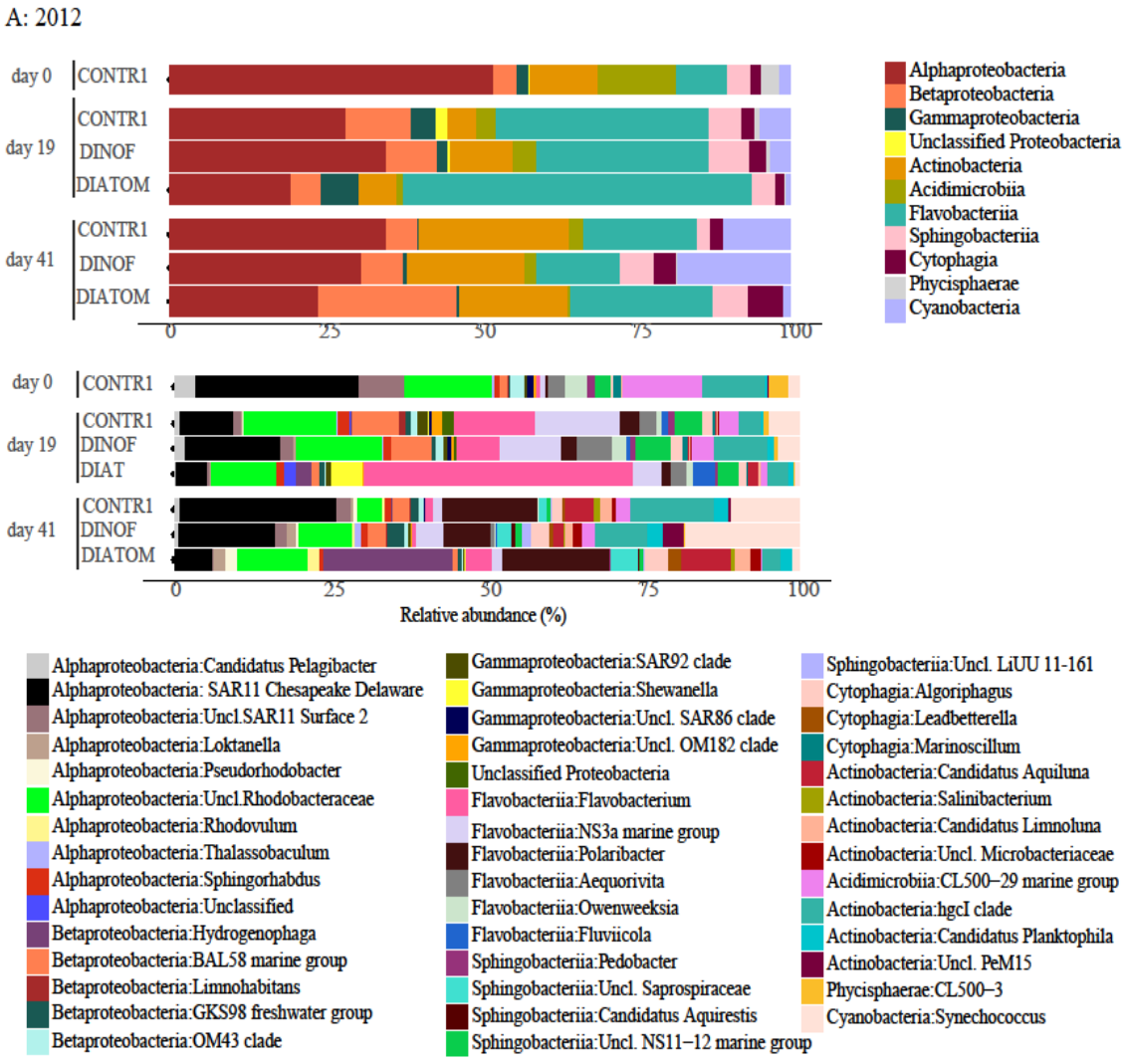
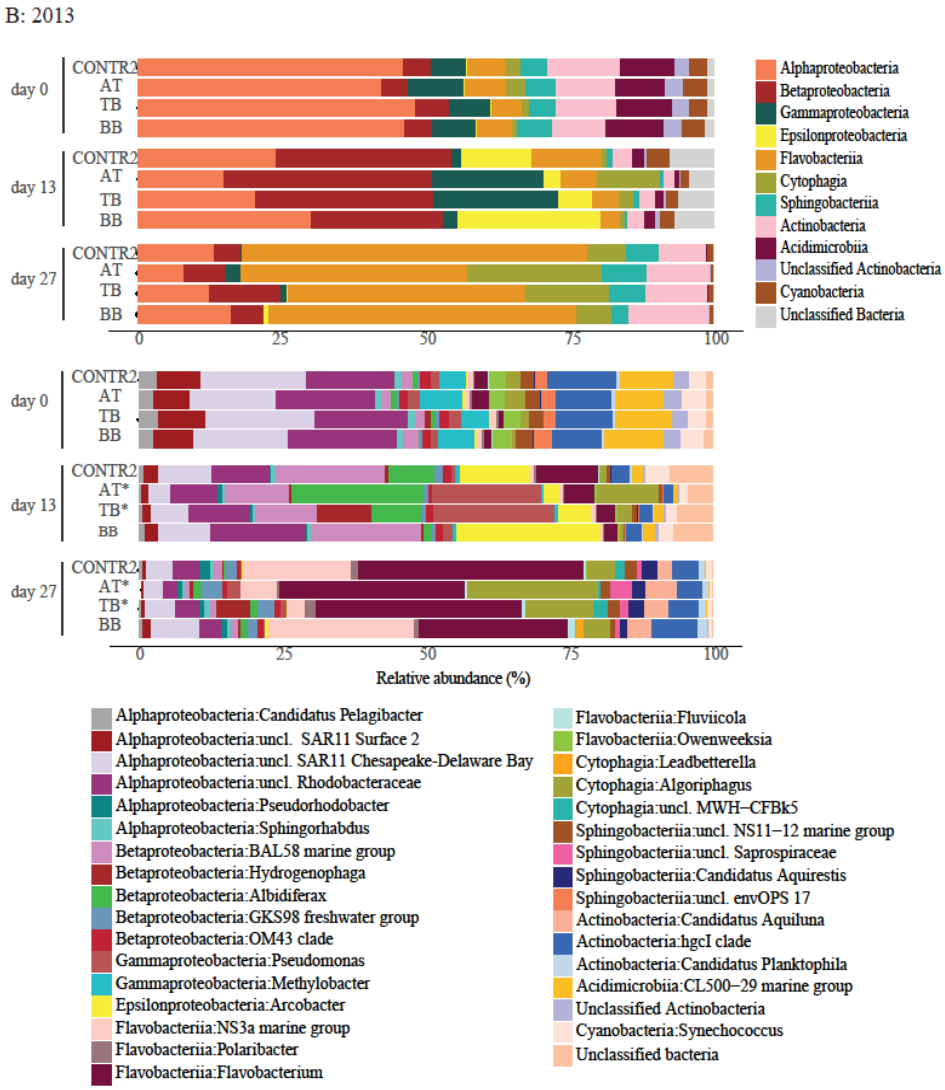


Figure 15 Bacterial community composition at the class- and genus level in the Gulf of Finland experiments; A) 2012 and B) 2013 on different sampling days: start (day 0), chlorophyll *a* peak (day 19 in 2012 and day 13 in 2013), bacterial production peak (day 27 in 2013) and end of the experiment (day 41 in 2012) in the controls and treatments.

Figure 15. (Continuation)



4.2.2 Bacterioplankton dynamics in the Baltic Sea: production, abundance, respiration and community composition (II)

In general, the BPL was higher in the cold spring bloom found in 2013 than in the other cruises, attaining values > 0.62 µg C L^{-1} h^{-1} during the Decline phase in the GoF and following the high PP tendency of this year (Fig. 16A). The BPL and BPT values were also higher in the warmer 2014 cruise during the Decline and Postbloom phases, with BPT values > 1.5 µg C L^{-1} h^{-1}, whereas in the other warmer cruises (2015 and 2016), the BSP was < 0.3 µg C L^{-1} h^{-1}. In contrast, the BA was higher in the low productive years (BSP and PP) and largely in 2015, with values > 4.8 x10^6 cells mL^{-1} (Fig 16B).

Overall, the BPT:PP and BPL:PP ratios were low on all the cruises, but with slightly higher values in the 2014 cruise and at the northernmost stations in the BoB (Table 5).

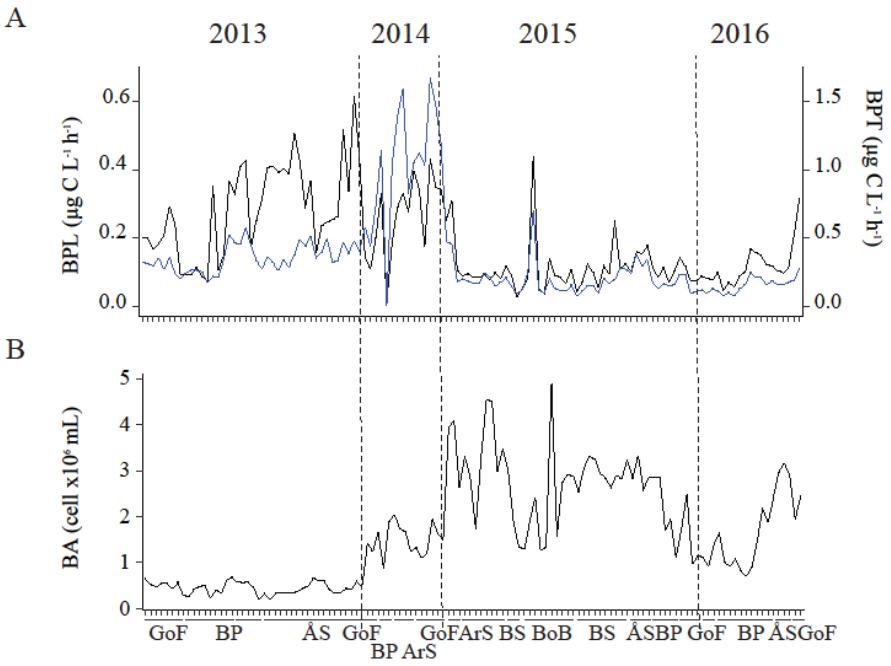


Figure 16. Heterotrophic secondary production A) based on leucine incorporation (BPL, black) and on thymidine incorporation (BPT, blue), and B) bacterial abundance (BA) dynamics on the various cruises in the Baltic Sea. The x-axes indicate the stations (126) with the subbasins covered on each cruise.

The BR, estimated in 2014 (six stations), 2015 and 2016, ranged from 0.05 to 2.78 µg C L^{-1} h^{-1}, being higher during the Peak/Decline/Postbloom phases but without significant differences between bloom phases (Fig. 17; Kruskal Wallis, $p = 0.57$).

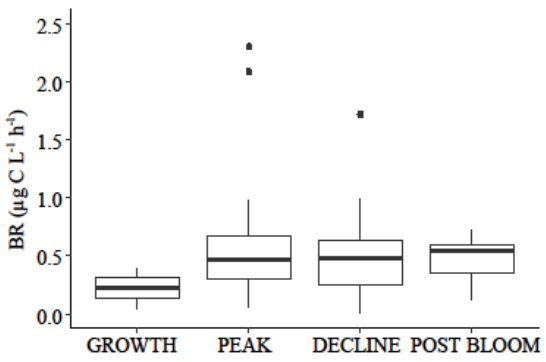


Figure 17. Bacterial respiration (BR) estimated as carbon dioxide production in the stations sampled (six in 2014, 2015 and 2016) in the varios phytoplankton bloom phases on the Baltic Sea cruises.

The bacterial community was clustered into two main groups, based on their richness and composition: a group associated with the cruises in 2013−2014 with high diversity and high relative abundance of Gamma-Betaproteobacteria, Bacteroidetes (Sphingobacteriia, Cytophagia and Flavobacteriia) and Actinobacteria, and a second group associated with the cruises in 2015−2016, and lower diversity and higher relative abundance of Alphaproteobacteria (SAR11 and Rhodobacteraceae), Acidimicrobiia, Phycisphaerae (2015) and Cyanobacteria (Fig. 18). Alphaproteobacteria dominated the bacterial community during the cruises, largely by SAR11 in 2015 (> 60 % of the OTUs), whereas some of the Alphaproteobacteria consisted of Rhodobacteraceae (genus *Gemmobacter*) on the other cruises (Fig. 19). Cyanobacteria were, in general, present in the warmer stations, with high relative abundance of *Synechococcus* sp. and also *Aphanizomenon* sp. in the 2016 BP subbasin. The phylum Phycisphaerae, represented by the genus CL500-3, was low in general, but comprised ~8 % of the relative abundance in the northern Gulf of Bothnia (BS and BoB). Bacteroidetes were also present at all the sampling stations and largely in the most productive years. The class Flavobacteriia dominated the community, which comprised on average ~ 25 % of the total relative abundance. *Flavobacterium* was the most representative genus within Flavobacteriia and contributed 8−12 % to the total relative abundance during the Peak/Decline phase in 2013 and 2016. There were other Flavobacteriia genera, such as *Owenweeksia* in the Growth phase and *Polaribacter, Fluviicola* and the NS3a marine group in a more advanced bloom, contributing to the high relative abundance of this group. Gammaproteobacteria, represented mainly by the genus *Crenothrix*, contributed between 10 % and 19 % to the relative abundance, but was restricted to the GoF subbasin during all the cruises and the ÅS subbasin in 2013 and 2016. The class Actinobacteria showed higher relative abundance in the 2014 cruise, largely by the hgcI clade (> 10 % of the OTUs), and the class Acidimicrobiia was represented by the CL500-29 marine group (> 5 % of the OTUs).

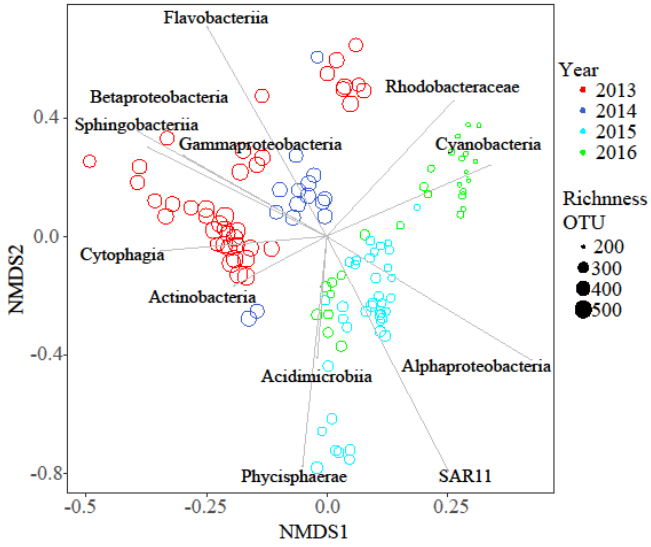


Figure 18. Nonmetric multidimentional scaling (NMDS) plot based on Bray-Curtis distances of bacterial community structure on the four cruises in the Baltic Sea. The colour-code indicates the stations sampled in each year and the dot size is the OTU richness. The vectors indicate the changes of the main bacterial groups, based on the dissimilatory distances. N = 122, stress = 0.134.

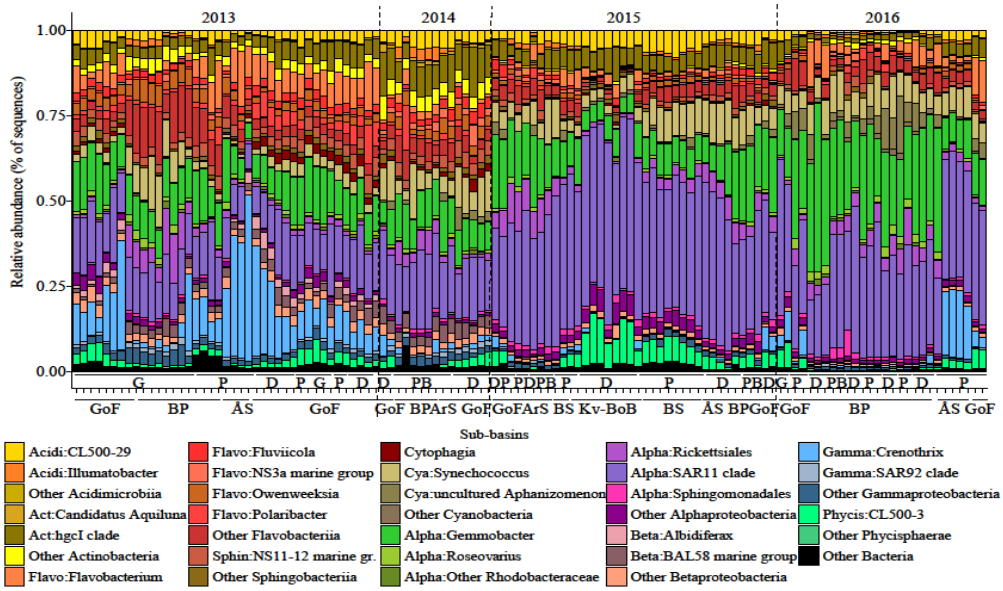


Figure 19. Bacterial community composition, measured as percentage of the relative abundance, along the stations sampled in the various subbasins and phytoplankton bloom phases during the four cruises (2013−2016). The legend present the main bacterial classes found: Acidimicrobiia (Acidi), Actinobacteria (Act), Flavobacteriia (Flavo), Sphingobacteriia (Sphingo), Cytophagia, Cyanobacteria (Cyano), Alphaproteobacteria (Alpha), Betaproteobacteria (Beta), Gammaproteobacteria (Gamma) and Phycisphaerae (Phycis).

4.2.3 Bacterial community structure in the Humboldt Current System experiment (III)

The BCCs in the water collected (day 0) at the three locations ('North', 'Central' and 'South') in the HCS were similar (Fig. 20). At the end of the experiment (day 12), the bacterial community differed between

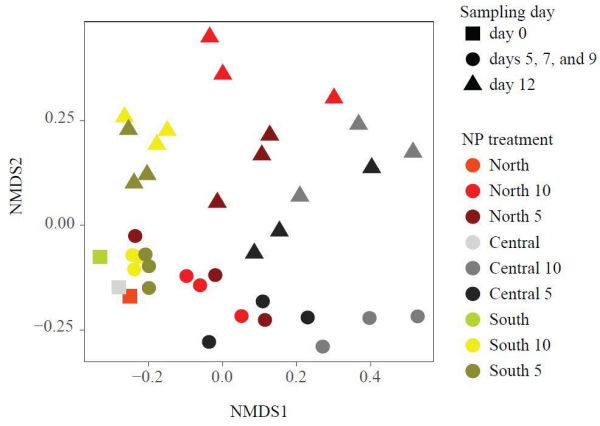


Figure 20. Non-metric multidimensional scaling (NMDS) plot based on Bray-curtis dissimilatory distance of the bacterial community sampled in the three communities 'North', 'Central', and 'South', with their corresponding nitrogen:phosphorus treatments (NP 5 and NP 10) indicated by colour, and on the three sampling days indicated by dot shape: start of the experiment (day 0), Chlorophyll *a* peak (day 5 in 'North', day 7 in 'South' and day 9 in 'Central'). N = 3 and stress value = 0.15.

the three locations (PERMANOVA, $p < 0.001$), being more dissimilar in the bacterial community of the location 'Central' than 'South'. However, no NP treatment effect was observed on the BCC (PERMANOVA, $p = 0.06$).

The water collected was dominated by the class Alphaproteobacteria, largely by genera from the family Rhodobacteracea (*Amylibacter, Loktanella* and *Planktomarina*), which contributed > 30 % to the relative abundance of the total community, and by the class Flavobacteriia (genus *Polaribacter,* Fig. 21A, B). This class showed higher relative abundance in 'North' (> 34 %) compared to the other locations, but other genera were present in 'South', such as *Formosa* and the NS4- and NS5 marine groups, which were absent in the locations dominated by diatoms (Fig. 21B). Cyanobacteria (genus *Synechococcus*) also contributed > 15 % to the relative abundance in 'Central' and 'South', while Gammaproteobacteria contributed > 10 % in 'North' and 'South' (Fig 21A).

At each of the the Chl *a* peaks (different date for each location), a similar bacterial community was still present, with the exceptional increase (25−40 % of the OTUs) in Sphingobacteriia (genus *Lewinella*) in 'Central'. At the end of the experiment, Gammaproteobacteria (genus *Pseudoalteromonas*) increased > 20 % of the relative abundance in 'North 10' and 'Central 10', which replaced the high relative abundance of Flavobacteriia in 'North 10', decreasing to < 7 %. In community 'South', the genus *Candidatus Actinomarina* (Acidimicrobiia) was present during the experiment, but with low relative abundance (< 1.5 %) and increased at the end of the experiment by > 12 % in 'South 10'. *Synechococcus* was also > 10 % but in 'South 5'. Both Acidimicrobiia and Cyanobacteria were absent in 'North' and 'Central' at the end of the experiment.

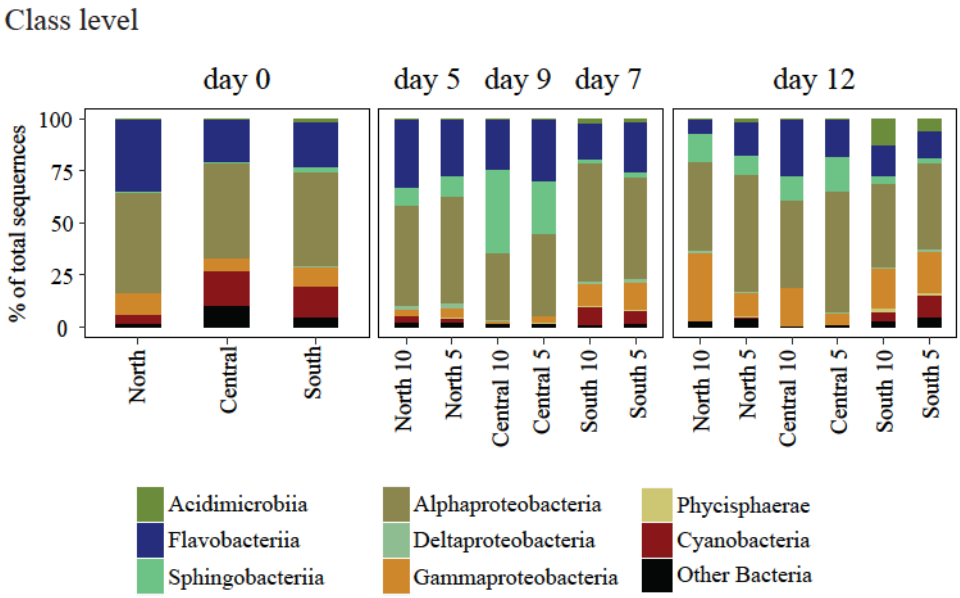
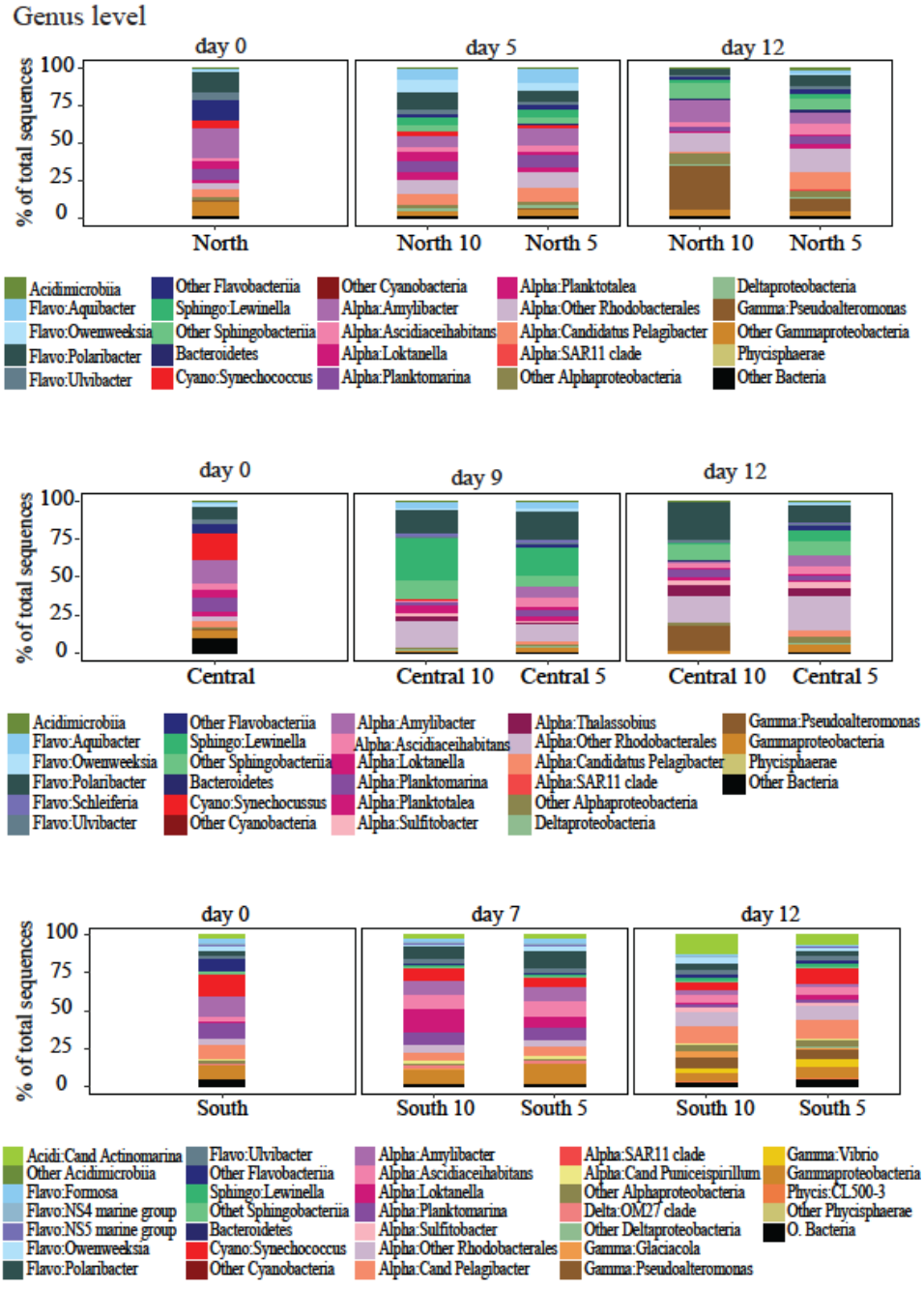


Figure 21. Bacterial community composition at the A) class and B) genus level in the Humboldt Current System experiment at the three locations 'North', 'Central' and 'South' and their respective nitrogen:phosphorus treatments (NP 5 and NP 10) on the three sampling days: start (day 0), Chlorophyll *a* peaks (day 5 in 'North', day 7 in 'South' and day 9 in 'Central') and end of the experiment (day 12). N = 1 day 0 and N = 3 on the remaining of the sampling days. Figure B is shown on the next page due to space limitation.

Figure 21. (Continuation)



4.3 Environmental factors shaping the bacterial community dynamics (II)

The bacterial community structure was partly explained by the salinity gradient observed during the cruises, from the more saline southernmost BP stations to the less saline northernmost BoB, and also by the temperature and the plankton community composition (Fig. 22). For instance, the high carbon biomass of certain diatom species (*A. taeniata, S. marinoi, T. levanderi* and *Chaetoceros* spp.) and also the presence of the microflagellate *Ebria tripartita* in the Peak/Decline phases of the bloom, as well as temperature and the POC:Chl *a* ratio significantly explained the segregation of bacterial communities between 2013−2014 and 2015−2016 observed. The taxonomic differences between the bacterial assemblages were significantly correlated with the overall differences between the plankton communities, a pattern that was consistently found throughout the four years of study (Spearman r values 0.63−0.79, Mantel test $p = 0.0001$, Table 7).

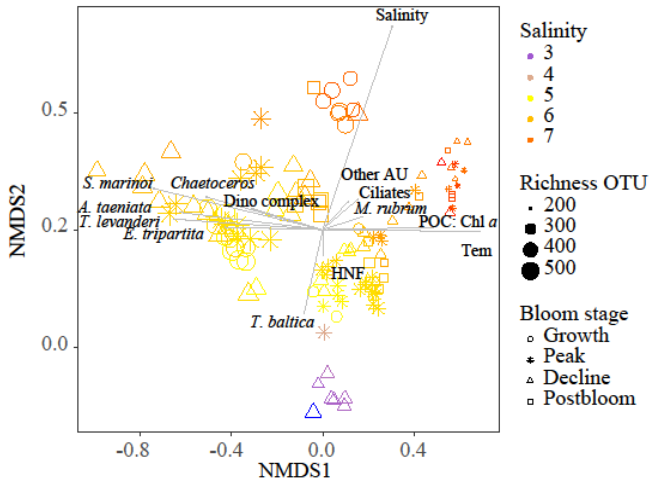


Figure 22. Non-metric multidimensional scaling (NMDS) plot of the bacterial community structure during the cruises in the Baltic Sea. The figure has emerged from the Bray-Curtis dissimilatory distance matrix, linked with the environmental and biological variables. The colour-code indicates the salinity gradient. The dot size indicates the richness of the operational taxonomic units (OTUs) and the shape indicates the phytoplankton bloom phase. The vectors indicate the significant environmental variables as *Achnanthes taeniata*, *Skeletonema marinoi*, *Thalassiosira levanderi*, *T. baltica*, *Chaetoceros* spp., dinoflagellate (Dino) complex, *Ebria tripartita*, *Mesodinium rubrum*, ciliates, heterotrophic nanoflagellates, other autotrophs temperature, particulate organic carbon:chlorophyll *a* (POC : Chl *a*) ratio and salinity. N = 122, stress = 0.134.

Mantel test	r value	p value
2013	0.754	0.0001
2014	0.627	0.0001
2015	0.783	0.0001
2016	0.784	0.0001

Table 7. Mantel test, based on Spearman correlations between the taxonomic dissimilarity matrices of the bacterial and phytoplankton communities, for each of the sampling cruises. The Spearman coefficients are indicated as r values and significance as p values ($p < 0.05$).

A more detailed exploration of the interactions between the main bacterial groups and environmental or biological variables showed the existence of two groups of bacterial taxa shaped differently by the environmental factors and phytoplankton groups studied (Fig. 23). These two bacterial groups coincided with the segregation observed in the bacterial community (community 2013−2014 and 2015−2016), based on the differences in their richness (Fig. 22). Whereas Bacteroidetes (Flavobacteriia, Cytophagia and Sphingobacteriia), Gamma- and Betaproteobacteria were positively linked with the diatom species as well as the presence of *Ebria tripartita*; the groups Rhodobacteraceae, SAR11 and the autotrophic Cyanobacteria were positively correlated with temperature, salinity and heterotrophic factors such as POC:Chl *a*, HNFs and ciliates. Phycisphaerae was specifically linked with the diatom *T. baltica.* The dinoflagellate complex correlated positively with the dominant taxa of 2013−2014 and also with Rhodobacteraceae from the segregated community of 2015−2016.

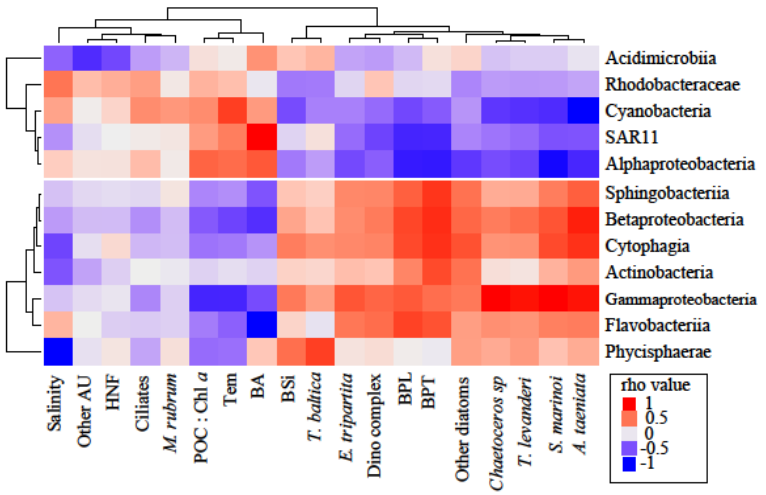
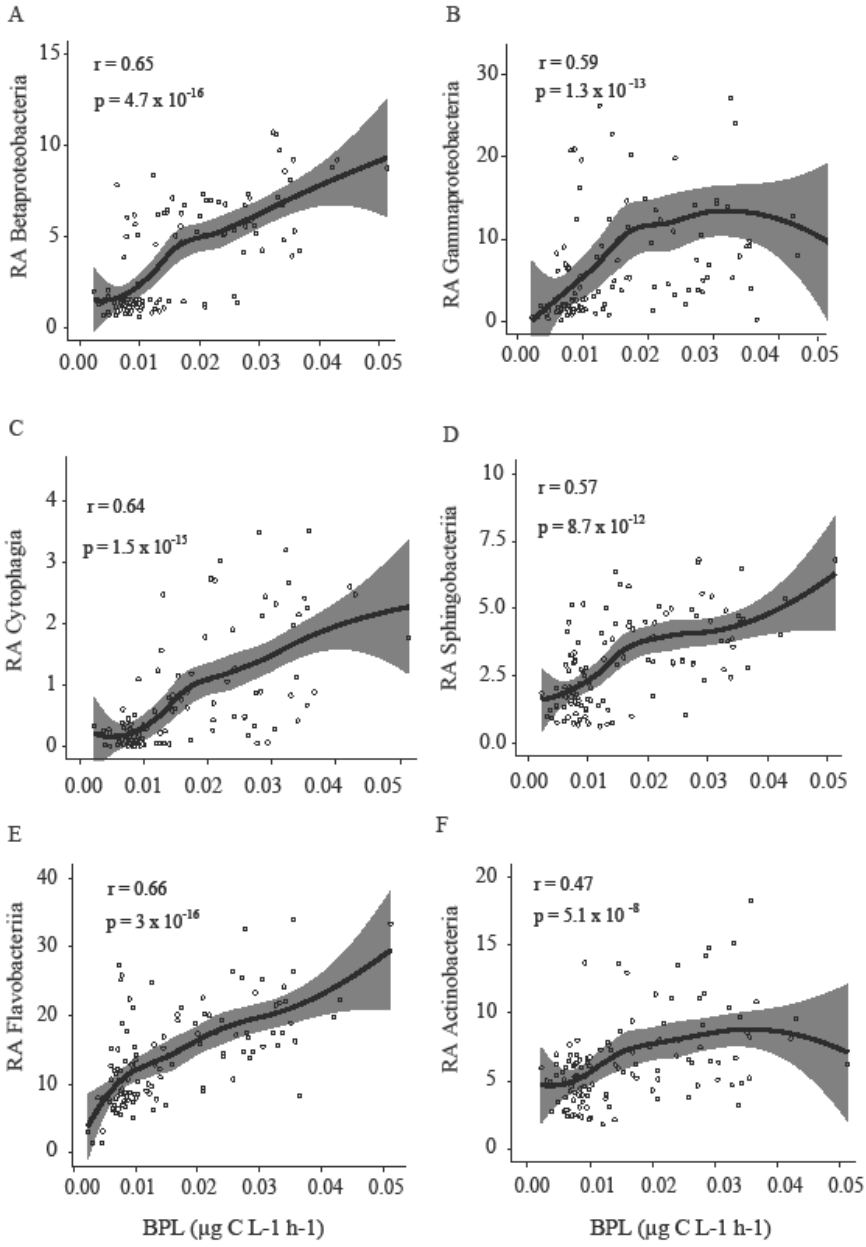


Figure 23. Heatmap based on Spearman correlation coefficients (rho values) between the main bacterial groups and the significant environmental and biological variables. The bacteria groups (rows) and dendrogram emerged by the clustering of the environmental factors, based on the similarities in rho values. The environmental factors (columns) are as follows: *Achnanthes taeniata*, *Skeletonema marinoi*, *Thalassiosira levanderi*, *Chaetoceros* spp., other diatoms, bacterial production based on thymidine and leucine, dinoflagellate complex, *Ebria tripartita*, *T. baltica*, biosilicate, bacterial abundance, temperature, particulate organic carbon:chlorophyll *a* ratio, *Mesodinium rubrum*, ciliates, heterotrophic, other autotrophs and salinity.

4.4 Link between the bacterial community composition and productivity (II)

The bacterial community structuring found during the four years of the study impacted the community functioning: the bacterial assemblage formed by the bacterial taxa Beta-, Gammaproteobacteria, Actinobacteria, Bacteroidetes, and more specifically Flavobacteriia, correlated positively and strongly with bacterial production rates, whereas Alphaproteobacteria (SAR11 in particular), was negatively correlated with bacterial heterotrophic production rates (Fig. 23). A detailed exploration of these trends (Table 8) showed that increases in relative abundance of groups such as Flavobacteriia, Cytophagia, Sphingobacteriia, Gamma- Betaproteobacteria and Actinobacteria, were associated with much higher BPT values and BPL (Fig. 24A−F), whereas the reverse was true for the abundance of SAR11 (Fig. 24G), which showed strong negative correlations with BPL incorporation rates. No correlation was found between Rhodobacteraceae, Phycisphaerae, Acidimicrobiia and BSP (Fig. 24H−J).


A
RA Betaproteobacteria
r = 0.65
p = 4.7 x 10-16
B
RA Gammaproteobacteria
r = 0.59
p = 1.3 x 10-13
C
RA Cytophagia
r = 0.64
p = 1.5 x 10-15
D
RA Sphingobacteriia
r = 0.57
p = 8.7 x 10-12
E
RA Flavobacteriia
r = 0.66
p = 3 x 10-16
F
RA Actinobacteria
r = 0.47
p = 5.1 x 10-8
BPL (µg C L-1 h-1)
BPL (µg C L-1 h-1)

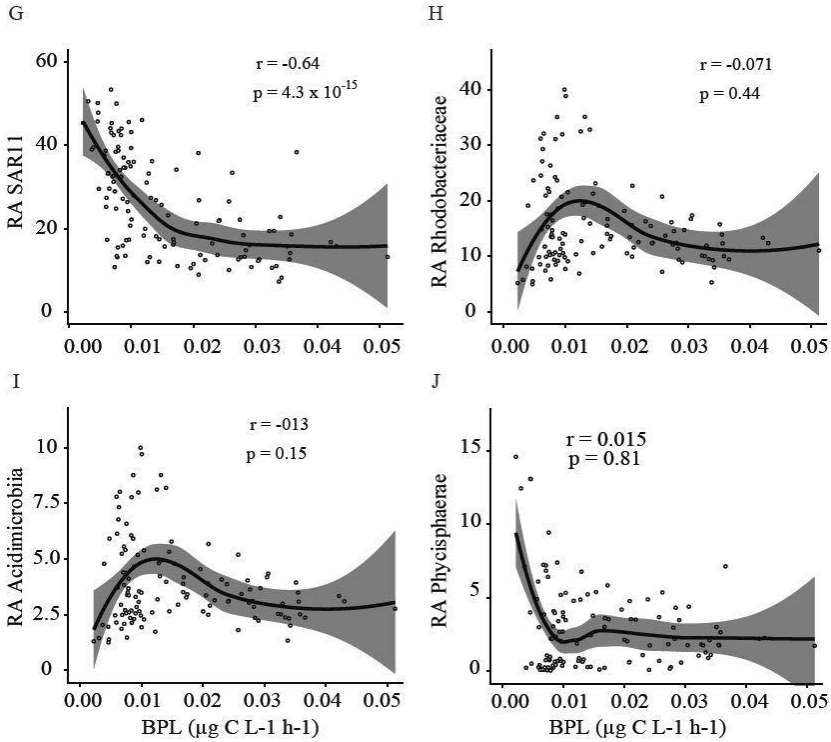


Figure 24. Spearman correlations between the bacterial productions measured as leucine uptake (BPL) and the relative abundance in percentage of A) Betaproteobacteria, B) Gammaproteobacteria, C) Cytophagia, D) Sphingobacteriia, E) Flavobacteriia, F) Actinobacteria, G) SAR11, H) Rhodobacteraceae, I) Acidimicrobiia and J) Phycisphaerae during the Baltic Sea study. Rho values and p values are presented in the figure. N = 122, $p < 0.05$.

Spearman Correlation	BPT rho value	p value
SAR 11	-0.636***	< 0.0001
Rhodobacteraceae	-0.006	0.509
Phycisphaerae	-0.017	0.850
Acidimicrobiia	0.105	0.252
Actinobacteria	0.662***	< 0.0001
Betaproteobacteria	0.706***	< 0.0001
Gammaproteobacteria	0.520***	< 0.0001
Sphingobacteria	0.687***	< 0.0001
Flavobacteriia	0.625***	< 0.0001
Cytophagia	0.698***	< 0.0001

Table 8. Spearman correlations (rho values) between the relative abundance of the main bacterial groups and the bacterial production thymidine (BPT) during the cruises in the Baltic Sea. N = 122. Statistically significant differences indicated by *** symbol, ($p < 0.05$).

5 DISCUSSION

The research for this thesis was conducted in the Baltic Sea and the Humboldt Current System off Chile, two different marine ecosystems that differ in their physico-chemical features, such as salinity and temperature. However, they have a common feature: the occurrence of phytoplankton blooms, dominated either by diatom and/or dinoflagellate communities, which allowed us to study the associated bacterial community dynamics during these highly productive events (I−III). To study that, I have combined experimental and field studies. The experimental studies aimed at investigating the possible consequences of changing phytoplankton communities on the bacterioplankton, both, structure and function. The strength of the experimental work is that it enables following the plankton dynamics under controlled environmental conditions (e.g. temperature and light) that may affect the plankton productivity and structure. Experiments may however not truly represent natural conditions, which is the strength of doing fieldwork. Sampling natural communities provides direct measurements of responses under realistic environmental conditions, but plankton dynamics are more difficult to follow. The results represent snapshots that may require additional data to be able to draw conclusions.

One of the uncertainties in our experimental design, collecting natural water as initial conditions, might come from different initial physiological status and biomass concentration of the primary producers. In the Tvärminne experiment (I), we defined the treatments by adding inoculums of phytoplankton cultures based on the measured Chl *a* concentration. The additions were higher in 2012 than in 2013, to ensure the shifting of the phytoplankton community, which also caused the differences between the 2012 treatments. These additions could have been made based on the carbon content of the phytoplankton, but this method for carbon determination is more time-consuming than using the Chl *a* concentration as reference. In any case, the added biomass was minimal compared with the biomass at the Chl *a* peaks (between 1,6% and 0,06% based on Chl *a* concentrations), and the same can be very likely applied to the plankton carbon biomass. Thus, we believe that the effect of the volume added as inoculum, did not significantly affect the bacterial bloom phase.

In the Chile experiment (III), no addition of cultures was done, but the initial Chl *a* concentrations varied between communities. We could have been applied a different approach to adjust the initial concentrations by doing a pre-treatment (e.g. dilution of two communities to the lowest concentration) in order to have the same starting biomass concentration in the three communities. However, the manipulation would have also added other uncertainties in terms of handling effects (e.g. stress) in the diluted communities.

The field study (II) allowed us to investigate the natural response of the microbial communities in the Baltic Sea. We covered a large area, from the northern GoB to the southern BP, and collected samples from different bloom phases during the spring season from four consecutive years (n = 127). This provides a dataset to compare with the patterns observed in the experimental study (I). The number of samples in the different subbasins was uneven, *e.g.* the GoB was covered only during the 2015 cruise during the Peak and Decline phases of the spring bloom. The unevenly of the sampling in addition to the heterogeneity of this ecosystem, *e.g.* salinity and temperature gradients and the different phytoplankton bloom development, adds some uncertainty. Nevertheless, the combination of experimental and field work provides added value by combining the strengths of these two approaches.

5.1 Structure and dynamics of the phytoplankton blooms

5.1.1 Environmental drivers of the phytoplankton blooms

The phytoplankton community found at each experimental site differed in terms of community composition and bloom phase, due to the dissimilar environmental conditions found at the time of sampling at each location. For instance, the phytoplankton community in the water collected of the GoF experiment was in the Prebloom phase, with high inorganic nutrient concentration (nitrate and phosphate), low carbon biomass and low Chl *a* concentration. These characteristics were related to the reduced light availability under the ice cover, reflected in the absence of PP at the time of sampling. The

light availability, governed by the built-up stratification and longer day lengths, is the key factor for the onset of the phytoplankton bloom in the Baltic Sea (Stipa 2004). In the Baltic Sea field study, the phytoplankton blooms were, from Growth to a more advanced Postbloom phase, reflected by the higher Chl *a* concentration and carbon biomass of diatoms and dinoflagellates than at the start of the GoF experiment. In this case, the sampling was conducted later in the year (April/May), and we found lower inorganic concentration and higher temperature than in the GoF experiments, factors that shaped the phytoplankton community during the cruises. Overall, the phytoplankton bloom development in the Baltic Sea differs by the subbasins, starting in the southern BP in February and moving northwards to the BoB in May (Kahru *et al.*, 1990), with the occurrence of the peak at different times, *i.e.* in the GoF it occurs when the nitrate is depleted (Tamminen & Andersen 2007). Thus, in coupling the results from both studies in the Baltic Sea (experiments and field study), we covered all the phytoplankton bloom phases, at least in the GoF subbasin, which is known to be one of the most important areas in terms of new production as well as the subbasin with the highest Chl *a* values during the spring bloom (Fleming & Kaitala 2006).

The initial phytoplankton community at the three sampling locations in the HCS experiment was in different phases of its growth (austral summer), differing in the initial inorganic nutrient concentrations and the initial carbon biomass found at the time of sampling. The location 'North' was in a more advanced growth state, with higher carbon biomass (> 500 µg C L^{-1}) and lower inorganic nutrient concentration than the locations 'Central' and 'South, which were in the growth and intermediate phases, respectively. In the HCS, characterized by a complex system of currents, the development of the phytoplankton bloom is promoted by the upwelling inorganic nutrient-rich waters. The upwelling is regulated by the wind regime and the ENSO, and it is enhanced by the topography of this region (Thomas *et al.*, 2001, Troncoso *et al.*, 2003, Montero *et al.*, 2007). The initial phytoplankton communities at the three locations were likely affected by different intensities of the upwelling event at the sampling time.

Nevertheless, the phytoplankton blooms in both systems were mainly influenced by N availability, as indicated by the depletion of nitrate in the course of the experiments (I, III), and at some stations during the cruises in the advanced phase of the bloom (*i.e.* Decline/Post bloom), similar to previous studies in the Baltic Sea (Tamminen & Andersen 2007) and the Humboldt Current (Messié & Chavez 2015). In contrast, the phytoplankton bloom in the BoB was limited by P. This common feature of decreased N:P ratios in both systems is caused by different drivers: eutrophication in the Baltic Sea (Meier *et al.*, 2011a, Meier *et al.*, 2012) and expansion of the OMZ in the HCS (Codispoti *et al*., 2005). In both cases, P-rich waters with low N:P ratios were observed at the surface.

5.1.2 Phytoplankton community composition during the blooms

In the three studies, diatoms and/or dinoflagellates were the dominant phytoplankton groups. They either developed naturally (II, III) or were promoted by the addition of monocultures (I), allowing us to study the bacterial communities associated with these distinct phytoplankton functional groups. The large addition of the monocultures in the 2012 GoF experiment clearly affected the phytoplankton bloom dynamics towards the dominance of the species added (*Chaetoceros wighamii* and dinoflagellate complex) and also promoted the development of an early Peak bloom phase with the highest carbon biomass in the DIATOM treatment. In the 2013 GoF experiment, a similar diatom carbon biomass was found, but the community was dominated by the same species that prevailed in the water collected (*Thalassiosira levanderi*) and not the added cultures. However, lower Chl *a* and PP values were observed in 2012 than in 2013, likely due to the lower sampling frequency in 2012 having missed the real Chl *a* peaks. The typical diatom (*T. levanderi*, *T. baltica, Chaetoceros* spp*, Skeletonema marinoi* and *Achnanthes taeniata*) and dinoflagellate (*Gymnodinium corollarium*, *Biecheleria baltic*a and *Peridiniella catenata*) species occurring during the spring bloom in the Baltic Sea (Spilling 2007) were observed during the GoF experiments, except *P. catenata* (I), and in our field study. During the cold 2013 cruise, we clearly observed the natural co-occurring diatom and dinoflagellate bloom. This cruise recorded the highest PP and Chl *a* concentrations for the entire field study, indicating an ongoing bloom (by definition in this study, the Peak phase). In contrast, more heterotrophic organisms, such as ciliates and HNFs, together with higher POC:Chl *a* ratios, which normally indicate a more heterotrophic system (Vargas *et*

al., 2007), were found when the water was warmer. HNFs are commonly found in the Baltic Sea and are main predators of the picoplankton (Kivi *et al.,* 1996). Large ciliates (~ 40 µm) also peak after the spring bloom in the Baltic Sea and can constitute up to 85 % of the heterotrophic biomass of the micro- and mesozooplankton, indicating that they are the main predators of the nanoplankton (autotrophic and heterotrophic) during the spring season (Johansson et al. 2004). A diatom bloom dominated only by *T. baltica,* together with the high carbon biomass of *Melosira arctica,* was found in the BS subbasin, which has also been previously reported by other authors (Andersson *et al.,* 1996).

In the HCS experiment, diatoms (*Thalassiosira* spp) predominated in the initial communities of 'North' and 'Central, as typically occurs in upwelling regions, due to their rapid growth under nutrient-rich conditions (Reynolds 2006), whereas *Prorocentrum* sp. predominated in 'South'. Dinoflagellate blooms usually occur at this location, likely due to Maipo River discharge, which influences the hydrological conditions in this area (Wieters *et al.*, 2003). After the addition of the inorganic nutrients at the respective N:P ratios, the phytoplankton communities developed differently. In 'North' the growth was faster than in 'Central' and was still dominated by large diatoms at the end of the experiment, whereas in 'Central' part of the diatom community was formed by flagellates (nanophytoplankton). The dinoflagellate carbon biomass in 'South' decreased throughout the experiment and was replaced by smaller cells such as *Synechococcus* spp. and picoeukariotes, but also showed more ciliates and mesozooplankton than the other locations. Smaller cells with higher surface-to-volume ratio are normally favoured under low inorganic nutrient conditions and are supported by regenerated production (Edwards *et al*., 2012). In addition, the large carbon biomass of the micro- and mesozooplankton observed in 'South' likely controlled the phytoplankton growth (*Prorocentrum* sp.) at this site.

5.2 Bacterioplankton response to distinct phytoplankton communities

5.2.1 Bacterial community: structure and dynamics

The BCC of the three studies was clearly affected by the phytoplankton bloom dynamics in terms of both community composition and the bloom phase, likely due to the nature and lability of the DOM release by phytoplankton cells (Ducklow 1983, Nagata 2000). Changes in the availability of the DOM release can alter the bacterial community dynamics, which has been reported in both, experimental studies (Riemann *et al.*, 2000, Pinhassi *et al.*, 2004, Sarmento *et al.*, 2013), and field studies during phytoplankton spring blooms (Teeling *et al.*, 2012, Lindh *et al.*, 2015, Bunse *et al.*, 2016).

The class Alphaproteobacteria dominated all the bacterial communities in both ecosystems (Baltic Sea and Humboldt Current) at the sampling time, but differed in the proportion of its main groups, such as SAR11 and Rhodobacteraceae with some taxa included in the *Roseobacter* clade (I−III). The SAR11 clade is a ubiquitous bacterial group found in all marine habitats (Gasol & Del Giorgio 2000, Giovannoni & Rappé 2000, Morris *et al.*, 2002, Carlson *et al.*, 2009, Brown *et al.*, 2012, Laas *et al.*, 2015) and considered as 'oligotrophic bacteria', due to the small genome and cell size. Thus, this group predominates in oligotrophic systems (Giovannoni *et al.*, 2005, Alonso-Sáez *et al.*, 2007) or under Prebloom conditions (Andersson *et al.*, 2010, Teeling *et al.,* 2012, Chafee *et al.*, 2018), similar to our results in the water collected of the GoF experiments. Interestingly, SAR11 strongly dominated the bacterial community on the cruises with fewer diatoms during the peak of the bloom (cruises 2015−2016), indicating changes in the availability of the algal-derived DOM that clearly benefited these oligotrophic bacteria.

In contrast to SAR11, the *Roseobacter* clade is associated with phytoplankton blooms (Alonso & Pernthaler 2006, Buchan *et al.*, 2014) of both diatoms (Grossart *et al.,* 2005, Giebel *et al.,* 2011) and dinoflagellates (Prokic *et al.,* 1998), and thus it was present in the water collected in the ongoing phytoplankton bloom in the HCS experiment. However, no species of the *Roseobacter* clade were observed during the blooms in the field study or with only low relative abundance (*e.g. Loktanella*) in the GoF experiments. The higher salinity in the HCS likely benefited the occurrence of this clade, since this bacterial clade is commonly found in marine or more saline environments (Buchan *et al.*, 2005).

During the course of the phytoplankton bloom in some of our studies (GoF, HCS experiments and 2013−2014 cruises), the bacterial communities were largely dominated by the associated bacterial taxa that typically occur in the blooms, such as the phylum Bacteroidetes (Flavobacteriia, Cytophagia and Sphingobacteriia), Beta- and Gammaproteobacteria and Actinobacteria (Riemann *et al.,* 2008, Teeling *et al.,* 2012, Hugerth *et al.*, 2015, Laas *et al.*, 2015). However, their proportions differed, depending on the dominant phytoplankton group and the bloom phase. Bacteroidetes was present in both systems and, in general, Flavobacteriia was more relatively abundant during diatom blooms than during dinoflagellates blooms in all the studies (I−III). Within the Flavobacteriia, a succession was clearly seen in the Baltic Sea: *Owenweeksia* was more abundant in the early bloom phases, whereas *Polaribacter* predominated more in advanced phases of the blooms, indicating different substrate preferences during the blooms (Teeling *et al.*, 2012). Other genera, such as the NS3 marine group and *Flavobacterium,* which largely predominated in the Baltic Sea, were more abundant during the Peak/Decline phase of the bloom. The success of Flavobacteriia during phytoplankton blooms is mainly due to their ability to degrade HMW compounds, such as proteins, chitin and polysaccharides (*e.g.* laminarin), which are largely produced by diatoms (Cottrell & Kirchman 2000, Becker *et al.*, 2017). The flavobacterial NS3 marine group has been previously observed during dinoflagellate blooms (Fandino *et al.*, 2001, Yang *et al.,* 2015), similar to our results in the Baltic Sea, as has the NS4-5 marine group in the dinoflagellate community during the HCS experiment, indicating that dinoflagellates can promote the growth of certain bacterial taxa.

Our work supports that Gammaproteobacteria is an opportunistic bacterial group (Lindh *et al.,* 2015), but this group differed within and between the studies, depending on the various phases of the phytoplankton bloom. In the Baltic Sea, Gammaproteobacteria (genus *Pseudomonas*) peaked briefly in the 2013 GoF experiment at the Chl *a* peak, while on the cruises (genus *Crenotrhix*) it was restricted to the Growth/Peak/Decline phases of the bloom. Gammaproteobacteria was also largely associated with the high carbon biomass of diatoms, rather than dinoflagellates, and due to its occasional occurrence we could have missed it in the 2012 GoF experiment. Gammaproteobacteria was also present throughout the HCS experiment, and the genera *Pseudoalteromonas* and *Vibrio* (dinoflagellate community) increased by the end of the experiment. These genera observed in our experiments are strongly associated with diatoms (Amin *et al.*, 2012 and references there in). Overall, Gammaproteobacteria, as a typical copiotrophic bacterial taxon, occurs briefly and responds rapidly to the presence of available inorganic nutrients and organic compounds (Pinhassi & Berman 2003, Pernthaler & Amann 2005, Gómez-Consarnau *et al.*, 2012, Sarmento & Gasol 2012). Thus, the transient occurrence of Gammaproteobacteria in both experiments and field study also corresponds with their high capability for quickly responding to environmental disturbances (Lindh & Pinhassi 2018).

Although, *Roseobacter* clade (Alphaproteobacteria), Flavobacteriia (Bacteroidetes) and Gammaproteobacteria are considered the 'master recyclers' of algal-derived DOM (Teeling, 2012, Buchan et al. 2014), mainly associated with diatom blooms (Amin *et al.*, 2012, Laas *et al.,* 2015, Chafee *et al.,* 2018), they differ in their metabolic affinities. The variety of DOM produced by diatoms likely establishes bacterial niche partitioning during the highly dynamic phytoplankton blooms (Teeling *et al.*, 2012, Taylor & Cunliffe 2017). This was supported by the lower net PER observed under diatom dominance than under dinoflagellate dominance in the field study (II). Diatoms likely released DOC with higher lability that was taken up by bacteria more rapidly than that released by dinoflagellates (Lignell 1990). The interaction between diatoms and the associated bacterial community seems to be established through synergistic interactions via hydrophobic signalling molecules accumulated in the phycosphere or close to the cell membrane of the algae (Amin *et al.*, 2012). The accumulated molecules, *i.e.* vitamins, iron, and DOM are rapidly assimilated by these associated bacterial taxa, highly contributing to the carbon cycling in marine ecosystems.

Typical freshwater bacterial taxa such as Betaproteobacteria, Actinobacteria and Phycisphaerae, were also observed during the phytoplankton blooms and mainly in the brackish Baltic Sea region, similar to previous studies in this ecosystem (Herlemann *et al.,* 2011, Rieck *et al.*, 2015, Bunse *et al.*, 2016). Of these groups, only the acidimicrobiial genus *Candidatus Actinomarina* (phylum Actinobacteria) increased in the dinoflagellate community of the HCS experiment together with the cyanobacterial *Synechoccocus*. *Candidatus Actinomarina* belongs to the 'marine actinobacterial clade' (Mizuno *et al.,* 2015), which has

been previously reported in this system during non-upwelling season (Aldunate *et al.*, 2018). In addition, Actinobacteria have been associated with cyanobacterial-derived DOM (Hugerth *et al.,* 2015) and likely they benefited by the increase in *Synechococcus* sp. Overall, Actinobacteria increased in the decaying phytoplankton blooms in the three studies and largely during the late phytoplankton bloom of the 2014 cruise, which contrast with previous observations in the Baltic Sea where it occurs later in the year, *i.e.* autumn (Hugerth *et al.*, 2015, Lindh *et al.,* 2015, Bunse & Pinhassi 2017). The high-frequency sampling during the phytoplankton bloom likely allowed us to observe not only the increase in Actinobacteria, but also the short peaks of the opportunistic Gammaproteobacteria.

Interestingly, we observed the development of filamentous bacteria in both systems with diatom-dominated communities, likely a response to higher grazing pressure on the diatom-associated bacterial communities than on the dinoflagellates. The filamentous morphotype is a protective strategy against grazing (Hahn *et al.*, 1999). For instance, together with the increase in filamentous *Lewinella,* we observed an increase in heterotrophic dinoflagellates and decrease in *Synechococcus* sp. abundance by the end of the HCS experiment, indicating more grazing pressure on the picoplankton. The filamentous *Crenothrix also* dominated the gammaproteobacterial community during the cruises under diatom-dominating conditions, and largely in the 2013 cruise, which was the cruise that showed the lowest BA. However, the role of *Crenothrix* is unknown in this ecosystem, since it is a methane-oxidizer normally found in sediments with the capability for uptake of single-carbon substrates (Stoecker *et al.*, 2006).

5.2.2 Link between the bacterioplankton and the environment: phytoplankton as a key driver of bacterioplankton changes (II)

The differences between the ecosystems studied regarding the physicochemical environmental conditions and the dominant phytoplankton species impacting on the bacterial community structuring and dynamics were reflected in the occurrence of distinct bacterial taxa. Analysis of the effects of environmental disturbances on the bacterioplankton during the field study (II) further revealed the importance of salinity, temperature and high carbon biomass of some diatom species (*A. taeniata*, *Chaetoceros* spp., *S. marinoi*and *T. levanderi*). These were the main drivers explaining the segregation of the bacterial communities into two groups: one with more copiotrophic bacteria (2013−2014) and one with more oligotrophic bacteria (2015−2016). This is not surprising, since temperature and salinity are known to affect the bacterial community in this brackish system (Andersson *et al,* 2010, Herlemann *et al.*, 2011, Rieck *et al.*, 2015, Herlemann *et al.*, 2016). Interestingly, temperature emerged as one of the main drivers for the structure of both the phytoplankton and bacterioplankton communities, suggesting that the increases in water temperature predicted throughout the Baltic Sea (Meier *et al*., 2012, Thomas *et al*., 2017) may lead to dramatic changes in the planktonic assemblages inhabiting this vulnerable system.

The bacterial community structure observed during the cruises was supported by the strong correlations we found between the main bacterial taxa and the main environmental drivers. Positive correlations were found between the bacterial community dominated by Gamma- and Betaproteobacteria, Bacteroidetes and the diatoms mentioned above, corresponding to their preference for diatom-derived DOM, whereas they correlated negatively with salinity, temperature and the POC:Chl *a* ratio. In contrast, SAR11 correlated positively with temperature and the more heterotrophic variables and negatively with high carbon biomass of diatoms and dinoflagellates, confirming its oligotrophic condition. Phycisphaerae was clearly affected negatively by salinity and appeared strongly associated with the presence of the *T. baltica*. This bacterial taxon is known to occur in the less saline Gulf of Bothnia and is influenced by allochthonous DOM (Rieck *et al*., 2015).

5.2.3 Linking bacterial community composition and ecosystem functioning (II)

To understand the role of the bacterial assemblages in different ecosystems dominated by diatom or dinoflagellate communities, the BSP was measured in the Baltic Sea studies (I, II). In both experiments and field studies, we observed higher BSP rates in the diatom-dominated communities, coinciding with higher relative abundance of copiotrophic bacteria such as Betaproteobacteria, Gammaproteobacteria and

Bacteroidetes. In contrast, low BSP rates were observed in dinoflagellate-dominated communities associated with bacterial communities largely dominated by SAR11. These results are in accordance with a previous field study in the Baltic Sea during growing diatom- and dinoflagellate-dominated blooms in which higher bacterial production and larger proportion of Bacteroidetes were associated with diatom blooms (von Scheibner *et al.*, 2018). Interestingly, low BSP rates were also observed in the presence of the diatom *T. baltica* in the BS, which coincided with low relative abundance of Bacteroidetes and the absence of Gammaproteobacteria. Despite our studies not having been focused on the analysis of allelochemical production, *Thalassiosira* spp. negatively affect the BPL of Bacteroidetes (Wichard *et al.*, 2005, *Balestra et al.,* 2011) and the production of polyunsaturated aldehydes (PUAs) by diatoms can inhibit bacterial growth as well (Amin *et al.*, 2012). Our results also showed that the BPL values were higher than those reported by previous studies conducted at similar temperatures during spring in the Baltic Sea (Lindh *et al.,* 2015, von Scheibner *et al.*, 2018), indicating that the dominance of various phytoplankton groups compared in their studies likely provoked differences in bacterial activity. High BPT rates were also observed during the declining phytoplankton bloom on the 2014 cruise, which may indicate an actively dividing bacterial community (Shiah & Ducklow 1997), and were mostly related to the highest relative abundance of Actinobacteria on this cruise. Actinobacteria are known to be highly efficient in TdR uptake (Pérez *et al.,* 2010). These differing patterns were confirmed by strong and positive correlations between the copiotrophic bacterial taxa and the BPL and BPT, and by the negative correlations observed with SAR11. From the consistency and robustness of the patterns observed, the results allowed us to establish a link between the taxonomic composition and ecosystem functioning, regardless of the large spatial and temporal variability covered in our study. So far, studies that have explored these relationships are scarce (Lindh & Pinhassi 2018) or have yielded inconclusive results (Langenheder *et al.,* 2005, Lear *et al.*, 2014). Thus, these findings are essential for the understanding of microbial ecology, since one of its goals is to determine the relevance of microbial diversity and composition for driving community functioning.

Dinoflagellates also correlated positively with the copiotrophic community in the field study, which is not surprising, since diatoms and dinoflagellates co-occurred in the Baltic Sea and some bacterial taxa likely benefited by the presence of dinoflagellates, as mentioned above. From the low BSP observed in the GoF experiment (I) with the same dinoflagellate-dominated community as in the field study, we can suggest that other phytoplankton species (*i.e.* diatoms) may have contributed to the high BSP rates observed in the field study. But, unfortunately, the results of our study are not as conclusive regarding the contribution of dinoflagellates as that from the diatoms. The lack of a deeper identification within the dinoflagellate group (species level) during the cruises provoked the shortage of determining whether there is any contribution from one of the possible dominant species (*B. baltica* and *G. corollarium*) to the carbon cycling (DOM release and carbon flux). This issue will remain unclear, constituting an interesting research topic for further studies. Thus, we suggest the need of introducing in monitoring programs the identification at species level of the three dinoflagellate groups compiled in the dinoflagellate complex (*A. malmogiense*, *B. baltica* and *G. corollarium*) to elucidate their role in the Baltic Sea ecosystem. All these evidences indicate the possible distinct effect of certain diatom and dinoflagellate species/genera on the bacterial cycling of carbon mediated by the presence of specific bacterial taxa.

5.2.4 Bacteria-phytoplankton coupling in the Baltic Sea (I, II)

The high BPT/BPL:PP ratios observed in the Prebloom phase in the cold water collected in the GoF experiment indicates the existence of bacterial activity, although the temperature was < 1 °C, and likely due to availability of allochthonous substrate at the sampling time. However, the strong decline in the ratios in the phytoplankton bloom phase at 4 ºC contrasted with the much larger values in the bacterial bloom phase at 10 ºC. The low temperature during the phytoplankton bloom development likely caused the delay in the bacterial growth during the bacterial bloom phase. This bacteria-phytoplankton uncoupling during the GoF experiment accords with previous studies during early spring blooms (Lignell *et al.*, 1993, Hoppe *et al.,* 2008). Temperature, together with grazing and inorganic and/or organic nutrient limitations, is known to control bacterial growth in cold environments (Lignell *et al.*, 1992,

Kirchman *et al.*, 2009, von Scheibner *et al.,* 2018). These disturbances can also explain the decrease in BA during the phytoplankton bloom in the 2013 GoF experiment. In the field study, the bacteria-phytoplankton uncoupling (ratios < 0.08) also occurred during the phytoplankton blooms, although the temperature ranged from 1 ºC to > 6 ºC, except in the late and warm phytoplankton bloom in the 2014 cruise, in which the BPT:PP ratio was > 0.25 at some stations. In addition, we observed different ratios between the treatments in the GoF experiment during the bacterial bloom phase, being much larger in the DIATOM treatment (BPT:PP > 9 and BPL:PP > 4) than in the other treatments. Hence, the substantial contribution of diatoms to the high BSP rates, likely due to the higher DOM lability, together with temperature, may have resulted in a synergistic effect on the bacterial growth in the communities from our study, as suggested in previous studies (Pomeroy & Wiebe 2001, von Scheibner *et al.*, 2014).

5.3 Conceptual model of the microbial loop during the spring bloom in the Baltic Sea

In highly productive regions, such as upwelling or eutrophic regions and during spring blooms, the plankton community is dominated by diatoms that favour a short eukaryote-dominated food web (Ryther 1969, Legendre 1990), as suggested for the Baltic Sea ecosystem (Hagström *et al.,* 2001). In the Baltic Sea, the main loss process following the spring bloom is sedimentation, and a mismatch between the spring phytoplankton bloom and the summer zooplankton bloom is known to occur, due to low temperature during the spring (Johansson *et al.*, 2004). Thus, the microbial loop may also play an important role in the carbon transfer following the sedimentation, as demonstrated in other productive regions (Teira *et al.,* 2003, Vargas & González 2004, Vargas *et al.,* 2007). The energy transfer through the microbial loop seems to be equivalent to overpass of the herbivorous food web, as well as the sinking fluxes, in most of the open ocean and oligotrophic waters (Cole *et al.*, 1988, Wylie & Currie 1991, Legendre & Rassoulzadegan 1995, Calbet & Landry 2004). For instance, Troncoso *et al.,* (2003) reported the highest BSP values in the HCS, which correlated positively with the PP and negatively with temperature. This indicates that organic matter availability may control bacterial metabolism in this ecosystem. Thus, the bacterioplankton were supported by the phytoplankton-derived DOM and constitute an important component in this upwelling ecosystem. High BSP rates indicate large assimilation of the labile DOM, which is repacked into microbial biomass and recycled to higher trophic levels. For this reason, a multivorous food web, combining the microbial loop and the traditional food chain, should be considered in most productive regions (Legendre & Rassoulzadegan 1995).

When considering the microbial loop as a link between different trophic levels, due to its role in the regeneration of nutrients and incorporation of the DOM pool in the carbon flux, it would useful to quantify more exactly the role of bacteria in the aquatic ecosystem. Thus, from our detailed study conducted in the Baltic Sea, I suggest a modified conceptual model of the microbial loop (Fig. 25), following the block diagram proposed by Ducklow 1983. In my model two different pathways, based on the various phytoplankton species predominating in the system, are highlighted. In pathway A, in which the phytoplankton community is dominated by diatoms, such as *A. taeniata*, *Chaetoceros* spp., *S. marinoi* and *T. levanderi*, that enhance the development of a productive bacterial community (Flavobacteriia, Beta- and Gammaproteobacteria, Actinobacteria) by the release of labile DOM. This pathway can promote a grazing cascade effect, starting from bacterivorous HNF feeding on the active bacterial cells that can potentially reduce bacterial cell numbers. The trophic cascade continues, with ciliates feeding mainly on different trophic levels such as HNFs and nanophytoplankton cells (preferably diatoms) during the decline of the bloom, and potentially on picoplankton (bacteria). In parallel, heterotrophic microflagellates can feed on phytoplankton; *e.g.* the algivorous *Ebria tripartita* preferably feeds on diatoms, as we observed in our study. Subsequently, the microzooplankton would act as the prey of the mesozooplankton, being dominated by rotifers and later by copepods (Johansson *et al.*, 2004). All these grazing steps can cause the release of inorganic nutrients and DOM by sloppy feeding and enhance the PP and also the bacterial activity of more copiotrophic bacteria by the inorganic nutrients and organic matter regeneration. In pathway B, the phytoplankton community is dominated by dinoflagellates or by the diatom *T. baltica,* which promote a bacterial community with lower relative abundance of copiotrophic

bacteria and/or is largely dominated by SAR11. This pathway would be less productive, due to the decreased DOM lability, which may already affect the carbon flux in the first step of the microbial loop. In addition, dinoflagellate-dominated communities may also decrease the carbon transfer to higher trophic levels by the reduction of ciliate predation on a less preferred food source. Overall, a reduction in carbon cycling through the microbial loop can be expected through this pathway.

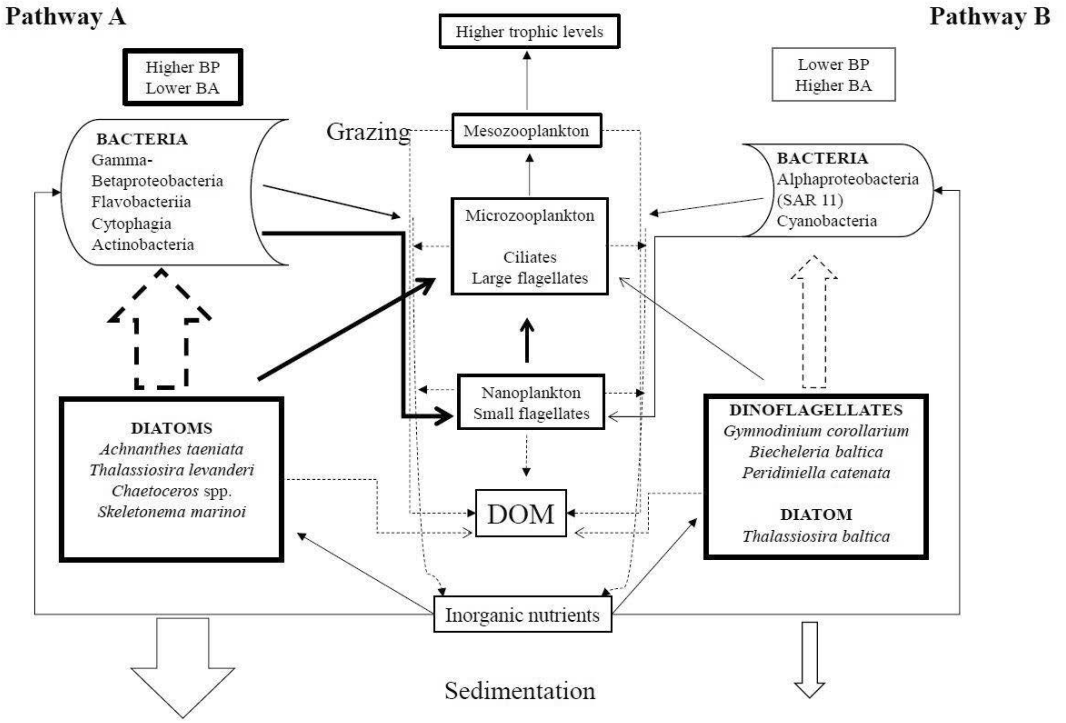


Figure 25. A modified box model diagram of the microbial loop from Ducklow (1983) applied to the Baltic Sea system during the spring bloom. The pathways (A, B) indicate the potential alternatives, based on the dominant phytoplankton communities. The heavy arrows indicate grazing and dashed arrows the release of inorganic and organic material.

6 CONCLUSION AND FUTURE PROSPECTS

This thesis highlights the importance of the phytoplankton community in shaping the bacterioplankton assemblage with distinct community structuring in the diatom and/or dinoflagellate communities, and observed in the Baltic Sea and the HCS of Chile. The succession the various bacterial taxa during the bloom events was likely caused by differences in terms of quality and/or quantity of the algal-derived DOM that caused the niche partitioning of the varying DOM utilization. Further studies of bacterial metabolism in the Baltic Sea confirmed the important role of specific diatom species in enhancing the development of copiotrophic bacterial communities characterized by their high levels of bacterial activity and which, consequently, are important for carbon cycling. These diatom species likely released high-quality DOM that favoured the carbon flux through the microbial loop. Since the DOC, as part of the DOM pool, is the major reservoir of organic carbon in marine systems and the most important carbon source for heterotrophic prokaryotes, further studies are necessary to understand the processes affecting the dynamics of the DOC pool in the global carbon cycle.

It is also important to remark that the role of dinoflagellates in the Baltic Sea, in terms of DOM release and contribution to the carbon flux, is unclear since they were not identified as single species and they co-occurred with diatoms. In addition, not all the diatoms contributed in the same extent to the bacterial productivity. Thus, we cannot draw a general pattern with the overall dominance of diatoms or dinoflagellates, but rather the presence of particular species. These findings indicate how crucial it is to identify the phytoplankton community to the class/genus level or even to species level when possible, to establish the link between phytoplankton and bacterioplankton and the link between the BCC and their functioning in the ecosystem.

Understanding the organic matter substrate preferences of the key heterotrophic bacterial taxa during phytoplankton blooms may give some insights into the role of heterotrophic bacteria in recycling of the DOC pool and in biochemical cycles. This can be performed by studying the chemical characterization of DOM released, either by their optical properties, molecular weight or chemical composition from the phytoplankton species of interest, such as the key diatom and dinoflagellate species that emerged in our study. On the other hand, it is also crucial to understand the functioning of the bacterial communities associated with the key phytoplankton species in using the DOM pool during the highly dynamic phytoplankton blooms. Thus, the methods used for gene expression, such as metatranscriptomics of natural bacterial communities, can unveil the metabolic processes occurring during phytoplankton blooms.

Regarding the effects of the environmental factors affecting microbial plankton community, temperature appeared as a main factor shaping both bacterial and phytoplankton communities in the field study. Interestingly, higher temperatures were not directly linked with higher bacterial production rates, highlighting the need to improve our knowledge of the effects of temperature on plankton community composition and production. The various phytoplankton species have different traits (Litchman & Klausmeier 2008), and the ongoing change, regarding global warming, in the phytoplankton community structure together with the increase in heterotrophic organisms clearly observed in the Baltic Sea, may have consequences for marine food webs and ecosystem functioning (Hansson *et al.*, 2013, Eggers *et al.*, 2014), *e.g.* bacterial activity and community structure with diatom- or dinoflagellate-dominant communities.

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